# Mass Cultivation of Algae and Photosynthetic Bacteria: Concepts and Application

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The cultivation of algae and photosynthetic bacteria for the production of useful resources has had a long and periodically active history. Aquatic raft farms of macroalgae have been an important part of the food industry in Japan for centuries. On the California coastline during World War I massive harvests of kelp were made to supply the war effort with the needed potash. The most recent surge in interest in the utilization of aquatic biomass was sparked by the energy crisis of the 1970's. It has led to an international effort for the development of new technologies with which the vast solar collecting area of the hydrosphere can be utilized for man's benefit.[3,16,39,53,59,61,73]

A broad range of photosynthetic organisms have been targeted for study, like kelp, porphyra and gelidium. For these species new research is primarily centred on expanding or improving on existing technologies for cultivation. A considerable volume of excellent literature is available on the work being done with these and other macroalgal species.[5,8,17,19,42,49,69,77,78] In contrast, photosynthetic micro-organisms have, historically, received considerably less attention, at least from the standpoint of practical application. There are some exceptions to this rule like the green algae, *Chlorella* and *Dunaliella*, and the blue-green alga, *Spirulina*. *Chlorella* and *Spirulina* have received a lot of attention as high priced health foods. This notoriety has spawned large-scale commercial ventures utilizing these species.[1,4,6,12,13,15,48,63,67,70] Use of *Dunaliella* to produce glycerol and β-carotene,[2] has also received considerable attention, but has not yet reached the level of commercial success enjoyed by the former two species.

In addition to the cultivation of aquatic photosynthetic organisms for specific foods or chemical by-products there is a more general interest in photosynthetic organisms for energy prodcution and wastewater treatment. The purification and stabilization of waste through photosynthesis has been successfully applied and could represent a potentially important source of nutrients through recycling.[11,20,22,26,39,50,51,63]

Another growing research interest is the conversion of aquatic biomass into fuels such as methane and alcohols.[18,61,62,78] This is usually accomplished through the fermentation breakdown of biomass by bacteria. Although most of these projects have not reached the stage of commercial application there are some preliminary indications that some of these systems could be feasible in the not-too-distant future.

The studies and projects discussed above are only a part of the diverse range of activities going on in this area of research and development. These represent some of the more traditional approaches to the utilization of aquatic biological resources. There are also a host of other innovative and often more speculative avenues of research which are being inves-

tigated. Our laboratory at the University of Miami, School of Marine and Atmospheric Sciences, has devoted more than ten years of research to such novel approaches in the use of photosynthetic organisms from the tropical marine environment. Rather than attempting to put together a detailed review of material which has already been superbly dealt with by other noted researchers (see references listed above), we shall devote the remainder of this paper to a description of the major research efforts carried out in our laboratory. Our efforts have been directed in three major lines of research, 1) the use of blue-green algae, as a feed in aquaculture, 2) the production of hydrogen gas by blue-green algae and photosynthetic bacteria and 3) the production of chemicals and biologically active substances by blue-green algae. All of the projects in these three categories conform to the major theme of our laboratory, namely the conversion of solar energy into useful resources through the use of microbial systems from saline environments.

## AQUACULTURE WITH NITROGEN FIXING BLUE-GREEN ALGAE

Our laboratory has explored the possibilities of nitrogen-fixing marine blue-green algae with respect to its potential as a direct feed source for aquaculture. Many conventional forms of aquaculture are inefficient in terms of tropic level energy conversion, because those rely on organisms that are too far from the ultimate energy source: solar radiation. Food and protein production systems based on direct utilization of primary producers such as marine blue-green algae, however, can circumvent such energy efficiency losses by eliminating one or more steps of the food chain.[32]

### Advantages of Using Nitrogen Fixing Blue-green Algae in Aquaculture

There are a number of advantages to nitrogen-fixing marine blue-green algae that make them uniquely suited to aquaculture applications.[32,33,39,40] Their most obvious attribute is the elimination of the considerable expense of supplemental nitrogen. Secondly, their nitrogen-fixing capability, which affords them the advantage of growing in a media without combined nitrogen compounds, in turn reduces or eliminates the growth of competing non-nitrogen-fixing micro-organisms. Toxic or pathogenic micro-organisms to fish and/or human beings are also curtailed by this combined nitrogen free environment. Thirdly, many blue-green algae have been shown to be high in protein and to contain the amino acids, lipids and other substances considered essential to the growth of fish. Fourthly, the nature of their cell wall gives them greater digestibility to aquaculture organisms in comparison with eukaryotic algae. Finally, some blue-green algae are tolerant to a wide variety of environments and can grow well in seawater. Thus, direct feeding of marine nitrogen-fixing blue-green algae to cultured organisms can have significant advantages.

We have done screening experiments with a number of potential aquaculture organisms using these strains of marine nitrogen-fixing blue-green algae with generally very good results.[40,45] Among these successfully grown animals have been rotifers, brine shrimp, penaeid shrimp and hybrid Tilapia fish. Our work with the latter organisms will be described herein as an example of our aquaculture research.

### Culture of Hybrid Tilapia with Nitrogen Fixing Blue-green Algae as a sole Feed Source

Hybrid Tilapia fish were chosen for this application for the following reasons: They are euryhaline, warm water fish and so, can be adapted to full strength tropical seawater, which is abundant and underutilized resource. They are extremely hardy, grow quickly, breed prolifically and produce good quality meat.

Short-term preliminary screening experiments with 91 strains of collection from this laboratory of nitrogen-fixing marine blue-green algae resulted in many blue-green algal

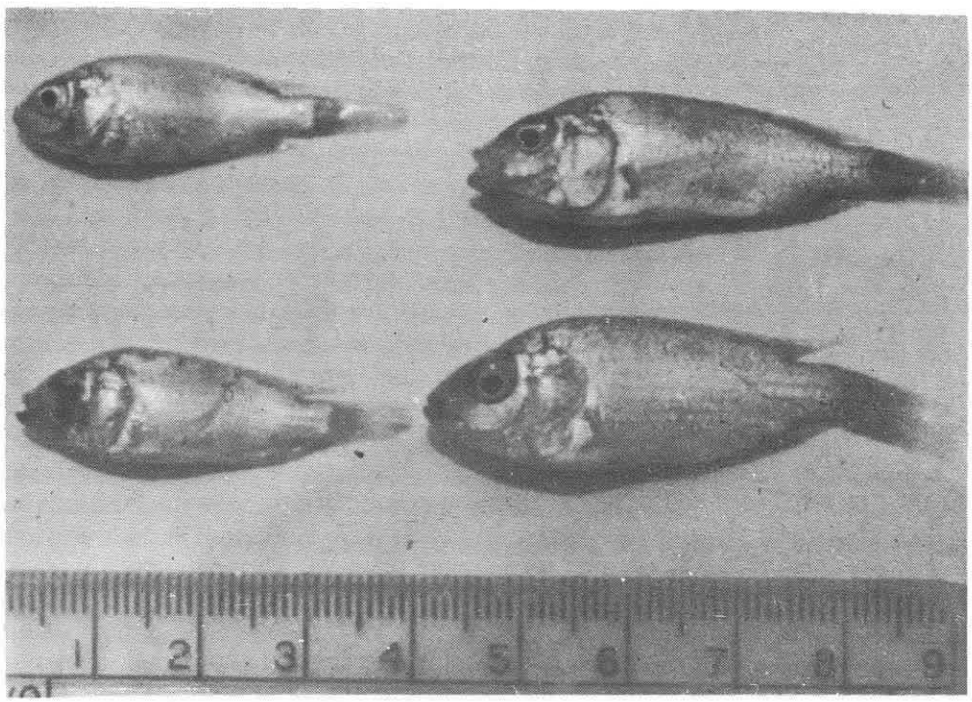

Fig. 1. Tilapia fish culture with nitrogen-fixing marine blue-green algae as sole food source. Fish on the above left are of initial size used for growth trials, and fish on the above right are of the final size after 5 days growth period (Murray and Mitsui, unpublished).

strains which supported the growth of Tilapia fingerlings (Table 1), and 22 blue-green algal strains which yielded very good specific growth rates for Tilapia fingerlings (more than 10%/day for a 5 day test period, examples are shown in Table 2 and Figure 1). Using 3 of the best of these strains as a sole food source, Tilapia fish culture was initiated in both in-

TABLE I

Overall Growth Response and Survival of Hybrid Tilapia-Fed Nitrogen Fixing Marine Blue-Green Algae in Preliminary Feeding Trials.[45]

| Blue-Green Algal Genus | Number of Strains of Blue-green Algal Tested | Strains Supporting Growth of Tilapia Fish | Fish Survival Rate % |
|---|---|---|---|
| *Nostoc* | 55 | 50 | 95 |
| *Calothrix* | 16 | 15 | 98 |
| *Anabaena* | 14 | 14 | 96 |
| *Scytonema* | 3 | 3 | 100 |
| *Nodularia* | 2 | 2 | 82 |
| *Hapalosiphon* | 1 | 0 | 0 |
| Combined | 91 | 84 | 95 |

door and outdoor systems and progress in that area was recently reported.[37] Efforts are now being focused on producing adult fish with these blue-green algal strains.

Blue-green algae were cultured in several 250 L clear fibreglass cylinders which contained a combined nitrogen free seawater based medium (Fig. 2). The cylinders were vigorously aerated with compressed air to keep the algal cells in suspension, and to provide atmospheric nitrogen and carbon dioxide. After establishing logarithmic growth, cultures were maintained at mid-log phase growth by the periodic addition of a sterilized combined nitrogen free seawater based medium provided by a sealed seawater sterilization system(Fig.2). The three blue-greenalgae showed high growth rates in both indoor and outdoor settings in these semi-continuous culture systems. Although blue-green algae generally prefer low light intensities for growth[54], these selected strains grew very well even in the high midday light intensities (e.g., 2,000 $\mu E/m^2/sec$). The blue-green algal biomass thus produced was directed via feeding conduits to the fish holding facilities (Fig. 2). The fish holding facilities consisted of 75 L glass aquaria indoors and 1200 L concrete tanks outdoors. Both are provided with aeration and filtered seawater flow through. Algal retention filters were affixed over all drain openings. Experimental fish were weighed on a weekly basis to monitor growth rates, and algal dry weights were monitored periodically to establish food conversion ratios. Feeding levels were on an ad libitum basis.

Fish, which were fed the 3 strains of blue-green algae, were successfully grown from the fry size to the young adult stage. Short-term growth rates for the fry (over 18%/day for a five day period) were extremely encouraging. More recent outdoor experiments comparing groups of fish which were fed ad libitum amounts of *Nostoc* sp. Miami BG 091911 and a formulated complete fish ration, respectively, also yielded very encouraging results. The two experimental populations of fish, which were held in adjacent tanks under identical conditions, displayed nearly identical growth rates. Over a 72 day period the algal fed fish grew at a specific growth rat of 4.35%/day and the formulated ration fed fish at 4.59%/day (Fig. 3). Both of these growth rates compare favourably with optimal growth rates reported for a variety of feeds in the literature.[7]

The results obtained indicate that the aquacultural systems, based on direct utilization

TABLE II

Nitrogen Fixing Marine Blue-Green Algal Strains which Exhibited High Percent Daily Weight Gains of Hybrid Fish *Tilapia*. 91 Strains of Blue-Green Algal were Tested for 5-6 Days. (After Murry and Mitsui[45])

| Diet Blue-Green Algal Genus | | Fish Initial Weight (g) | Fish Final Weight (g) | Specific Growth Rate (% per day) |
|---|---|---|---|---|
| *Nostoc* sp. | Miami BG 091911 | 0.320 | 0.745 | 18.41 |
| *Nostoc* sp. | Miami BG 050201 | 0.327 | 0.750 | 18.06 |
| *Calothrix* sp. | Miami BG 060201 | 0.415 | 0.758 | 16.25 |
| *Calothrix* sp. | Miami BG 095801 | 0.453 | 0.695 | 15.34 |
| *Anabaena* sp. | Miami BG 087101 | 0.347 | 0.688 | 14.67 |
| *Calothrix* sp. | Miami BG 027501 | 0.414 | 0.670 | 12.79 |
| *Calothrix* sp. | Miami BG 071301 | 0.386 | 0.691 | 12.36 |
| *Nostoc* sp. | Miami BG 081301 | 0.320 | 0.567 | 12.12 |

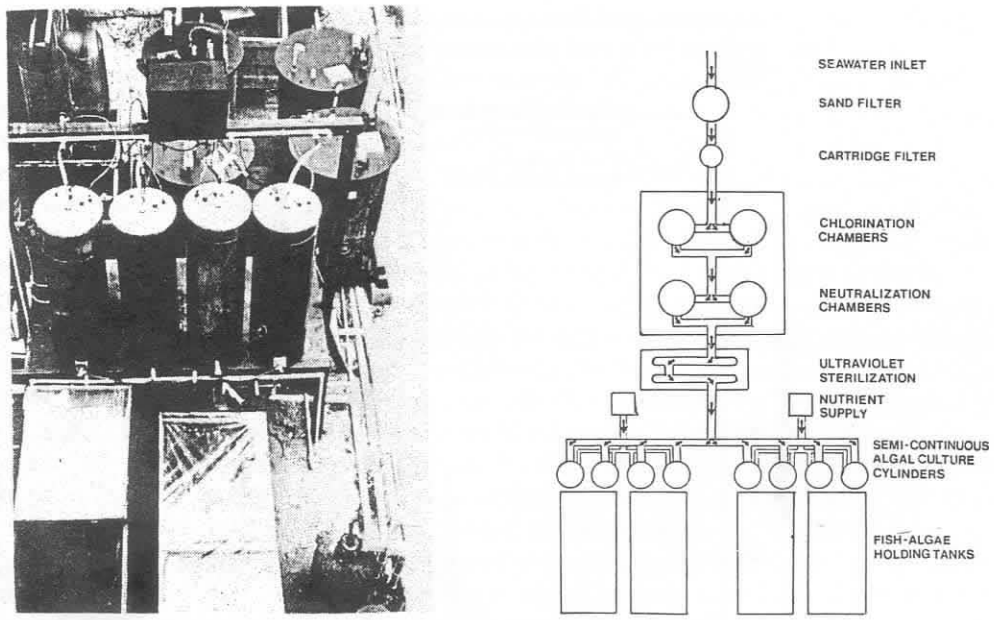


Fig. 2. Tilapia fish/nitrogen-fixing marine blue-green algal culture facilities (Gill, Sesco and Mitsui, unpublished). Four large tanks at top are a seawater sterilization system used to provide sterile seawater from semi-continuous culture of nitrogen-fixing blue-green algae in cylinders in centre on raised platform. Large tanks in foreground are fish holding facilities.

of marine nitrogen fixing blue-green algae which use only seawater and solar energy, can compare quite favourably (as described above) with the traditional aquaculture systems that are based on the use of the manufactured processed feed sources. Further experimentation is presently under way to support this contention.

## HYDROGEN PRODUCTION BY PHOTOSYNTHETIC MICROORGANISMS

In the early 1970's a number of laboratories in the U.S., Europe and Japan initiated research aimed at the use of biological hydrogen production for fuel production.[31,38] Now, a decade later, much of the fundamental basic research has been completed and this concept is ready to enter into the next stage of development, namely, testing the effectiveness of prospective designs of hydrogen production systems. This is, therefore, an opportune time to review some of the more important conclusions derived from the last ten years of research.

### Selection of Marine Blue-green Algae (Cyanobacteria) and Photosynthetic Bacteria for the Development of Hydrogen Production Systems

At the beginning of our involvement with biological hydrogen production in 1972, we hypothesized that the great diversity of species in the tropical marine environment could yield species uniquely well adapted for our applied goals. Essentially we were looking for strains of blue-green algae (cyanobacteria) and photosynthetic bacteria which exhibited both exceptionally high growth rates and hydrogen production capacity. During a broad sweeping survey of environmentally diverse habits over a ten year period throughout the tropical Atlantic thousands of samples were collected from which hundreds of microbial strains were isolated[27,29,30,38,41] (Fig. 4). Through several years of subsequent testing and ex-

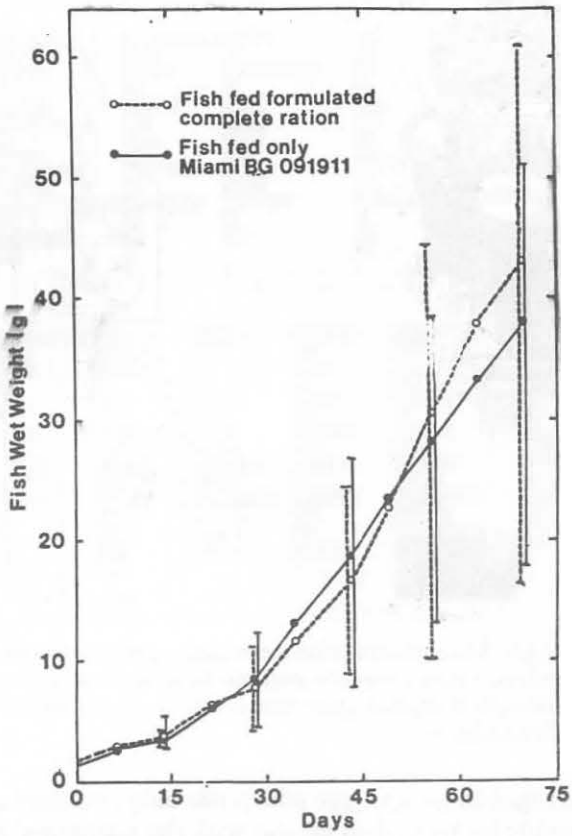


Fig. 3. Comparison of specific growth rates for two groups of Tilapia fish held in adjacent tanks under identical conditions and ad libitum feeding regimes. One group was fed a formulated complete fish ration and the other fed solely on *Nostoc* sp. Miami BG 091911 (Gill, Sesco and Mitsui, unpublished).

perimentation, a number of strains were earmarked as prime candidates for our ultimate goals. These screening experiments are focussed on the long-term accumulation of hydrogen, that is for days,[34,36,38] rather than short-term production. For applied systems development, stable hydrogen production for long periods of time and its accumulation in closed vessels is desirable. So far, over 150 strains of marine photosynthetic microorganisms have shown the capability for hydrogen photoproduction.

**Blue-Green Algal Hydrogen Production**

There are three basic types of blue-green algae; filamentous heterocystous, filamentous non-heterocystous and single-celled forms (Fig. 5). During our survey of hydrogen production capacity we ultimately selected one or two strains from each category for detailed experimentation. Based on the results of other researchers we initially suspected that heterocystous forms might be best suited for this application. This was based on the fact that the heterocystous found in this species provide the microaerobic environment needed for active hydrogen production to take place. As it turned out, heterocystous forms proved to have certain major disadvantages with regard to hydrogen production which make them less viable for these systems than the other blue-green algal types. The most serious drawback of these types of blue-green algae (heterocystous forms) is the existence of high up-

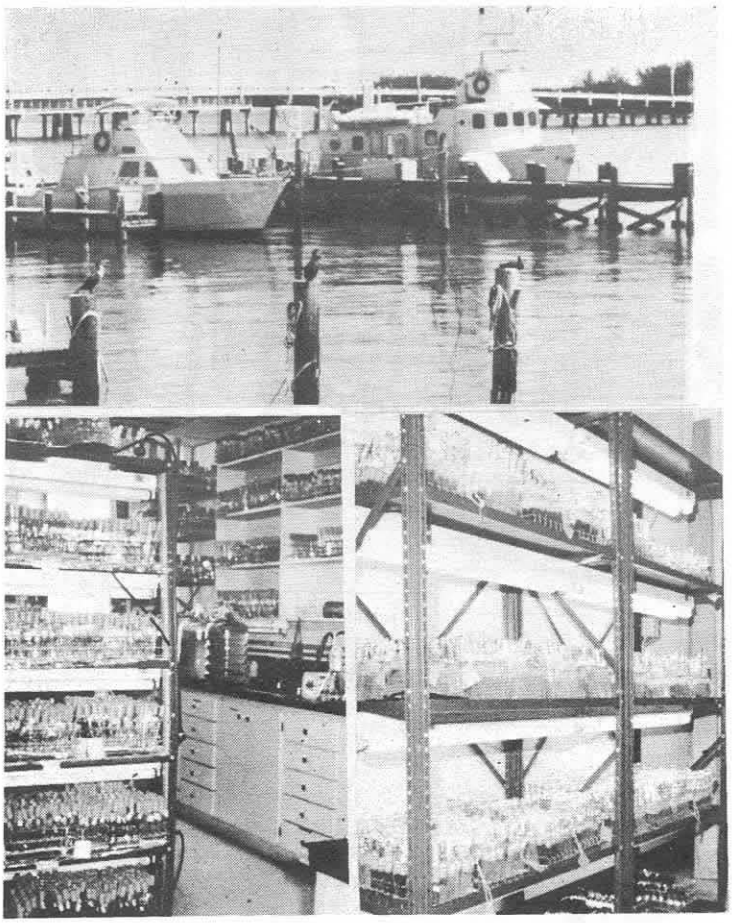

Fig. 4. Research vessels used for sampling of marine photosynthetic microorganisms. Collection of the marine blue-green algal strains (right) and marine photosynthetic bacterial strains (left) in Mitsui's laboratory

take hydrogenase (hydrogen consumption) activity. For example, the hydrogen production of the well-known heterocystous form of blue-green algae, *Anabaena cylindrica*, was short-lived, because the hydrogen accumulated was rapidly taken up again by uptake hydrogenase activity:[24,33,68] A similar pattern was observed for all of our own marine heterocystous isolates[41] including BG 047021 (Fig. 6). The way to overcome this problem is to continously remove the hydrogen as it is produced (Fig. 15). This may complicate the process and or may be too expensive to do in large-scale hydrogen production systems. In contrast, the non-heterocystous blue-green algae and some single-celled blue-green algal strains tested, exhibited no uptake hydrogenase activity.[23,41] (Fig. 6). This permitted hydrogen to accumulate in reactors until the concentration reached high enough levels to harvest (Fig. 15). Because of the problem of uptake hydrogenase activity our emphasis was shifted to the other two types of blue-green algae. Our most successful species have been the non-heterocystous filamentous *Oscillatoria* sp. Miami BG 7 and a single-celled nitrogen-fixing species *Synechococcus* sp. Miami BG 043511.

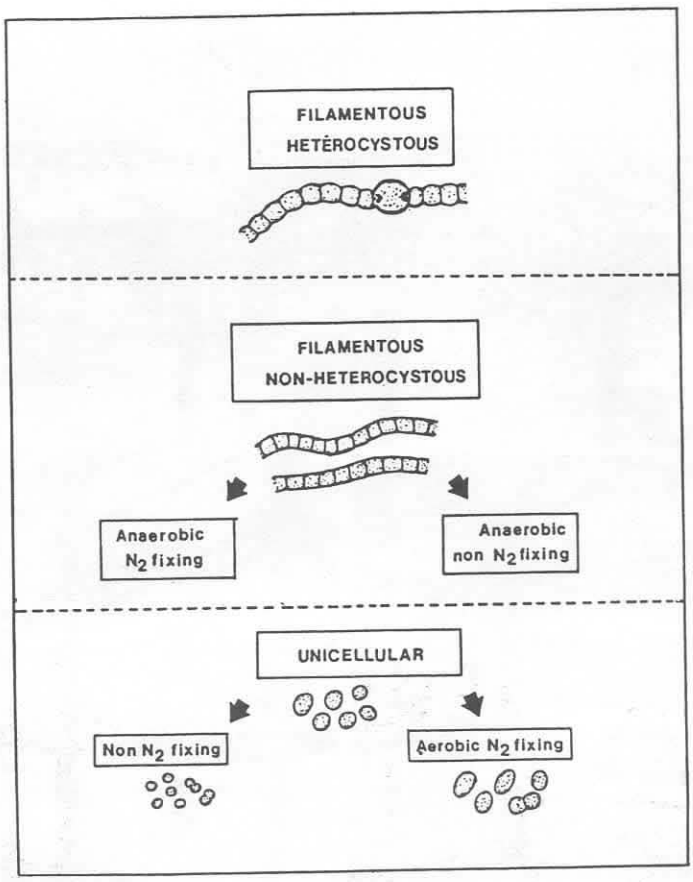


Fig. 5. Three major morphological forms of blue-green algae (Cyanobacteria)

**Two-Step Hydrogen Production System using Filamentous, Non-heterocystous Marine Blue-green algae and Salt Water as a Hydrogen Donor**

*Oscillatoria* sp. Miami BG 7 was isolated from the South Florida marine environment. (Mitsui and Rosner, unpublished). The two-step hydrogen photoproduction system developed with this strain is depicted in Figure 7. In the first step, cells were cultured in a combined nitrogen-limited medium. During this culture period, the concentration of intracellular hydrogen donor substance, that is, glycogen, increased dramatically, from an initial concentration of 20% to a final concentration of 65% of dry weight (Fig. 8). This creates a high cellular C/N ratio and resulted in the enhancement of nitrogenase synthesis. Meanwhile the concentration of photosynthetic pigments like chlorophyll and phycobilin, which are responsible for oxygen photoproduction, decreased significantly, thus minimizing oxygen inhibition of nitrogenase (or hydrogenase), (Fig. 9). Many green and blue-green algae are unable to produce hydrogen for sustained periods of time because the enzymes nitrogenase and hydrogenase, which are responsible for the terminal reaction of hydrogen photoproduction, are inhibited by photoproduced oxygen.

During the second step, the cultured cells were incubated under anaerobic conditions (argon atmosphere). Upon illumination, the accumulated intracellular glycogen was hy-

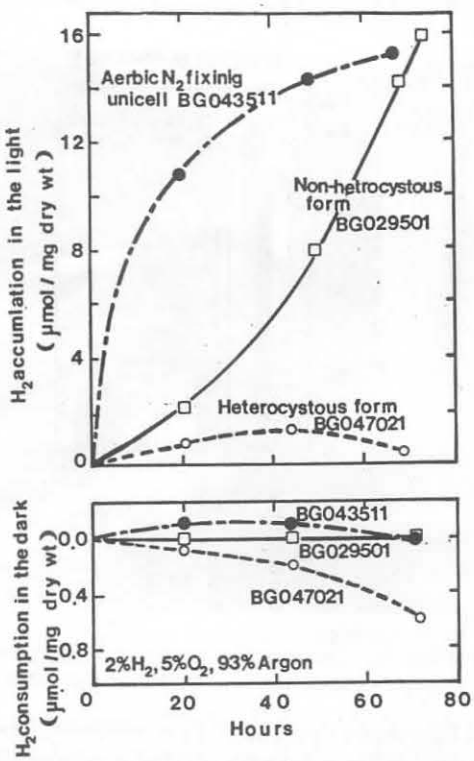


Fig. 6. Comparison of three forms of marine cyanobacteria; heterocystous filamentous, non-heterocystous filamentous, and aerobic nitrogen fixing unicellular, on hydrogen photoproduction and dark hydrogen consumption. Compared under similar conditions but not necessarily to reflect optimum conditions (Reddy and Mitsui, unpublihed)

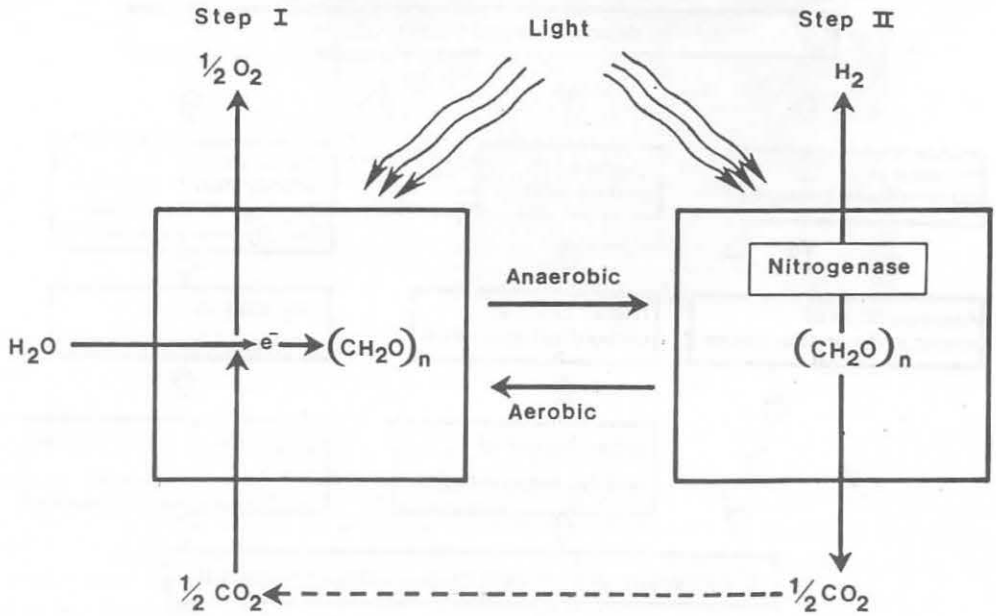


Fig. 7. Scheme for two-step hydrogen production from salt-water using filamentous non-heterocystous blue-green algae[41]

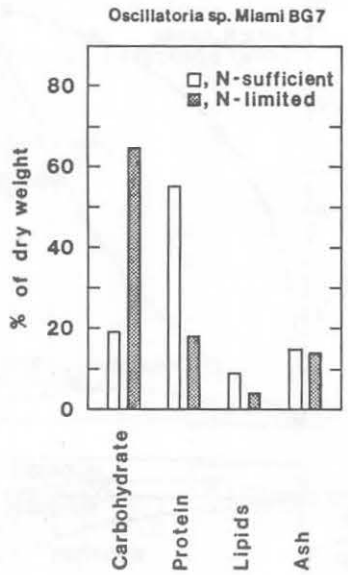


Fig. 8. Contents of various cellular substances in combined nitrogen sufficient and limited cultures of *Oscillatoria* sp Miami BG 7. (Kumazawa and Mitsui, unpublished and ref 41)

drolyzed to glucose and this was subsequently converted to hydrogen and carbon dioxide in a stoichiometric ratio of 2.[23] Experiments yielded a value of 9.8 as the molar ratio of hydrogen production to glucose consumption. In other words, 82% of the combustible energy held in the form of photosynthetically produced glycogen was converted to hydrogen

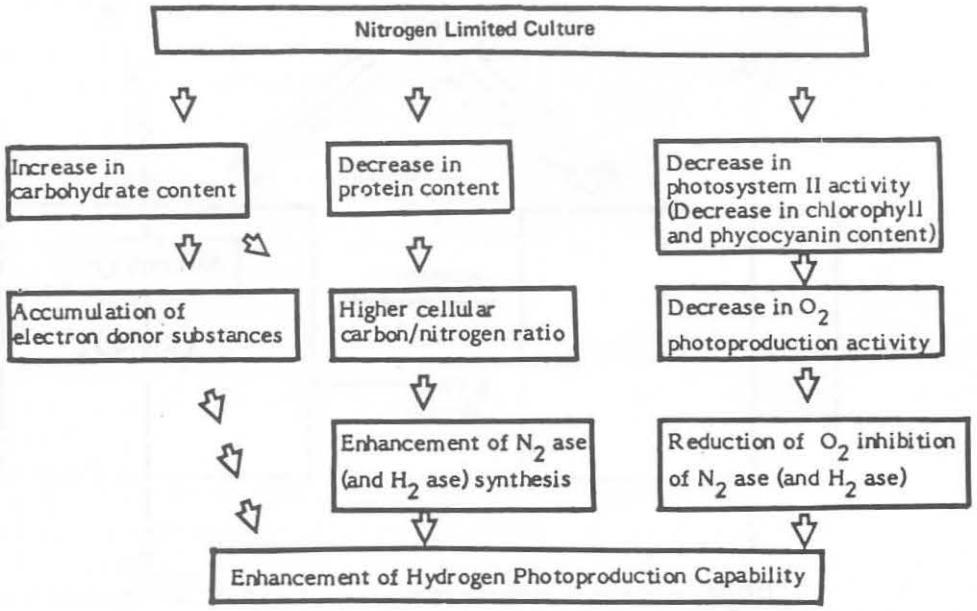
Nitrogen Limited Culture
Increase in carbohydrate content
Decrease in protein content
Decrease in photosystem II activity (Decrease in chlorophyll and phycocyanin content)
Accumulation of electron donor substances
Higher cellular carbon/nitrogen ratio
Decrease in $O_2$ photoproduction activity
Enhancement of $N_2$ ase (and $H_2$ ase) synthesis
Reduction of $O_2$ inhibition of $N_2$ ase (and $H_2$ ase)
Enhancement of Hydrogen Photoproduction Capability

Fig. 9. Mechanisms of enhancement of hydrogen photoproduction capability in combined nitrogen limited culture of blue-green algae[23]

(Kumazawa and Mitsui, unpublished).

Equations for step 1 and step 2 and the overall reaction are shown below. Overall inputs in these two steps are water and light and overall outputs are hydrogen and oxygen.[41]

Step 1 : Aerobic Reaction

$$12H_2O + 6CO_2 \xrightarrow{\text{light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

$$nC_6H_{12}O_6 \longrightarrow \text{glycogen} + (n-1)H_2O$$

Step 2 : Anaerobic Reaction

$$\text{Glycogen} + (n-1)H_2O \longrightarrow nC_6H_{12}O_6$$

$$C_6H_{12}O_6 + 6H_2O \xrightarrow{\text{light}} 6CO_2 + 12H_2$$

Overall Reaction:

$$H_2O \xrightarrow{\text{light}} H_2 + \tfrac{1}{2}O_2$$

These 2 steps can be efficiently recycled. Direct comparison of hydrogen photoproduction in closed vessels between the non-heterocystous blue-green algae *Oscillatoria* sp. Miami BG 7 and a well-known hydrogen producing heterocystous blue-green algae, *Anabaena cylindrica*, is shown in Figure 10. These blue-green algae werecultured and treated identically, and the same amount of blue-green algae was used for the comparison of hydrogen production. As described above, while *Anabaena cylindrica* could not accumulate hydrogen in closed vessels, *Oscillatoria* sp. Miami BG 7 produced and accumulated hydro-

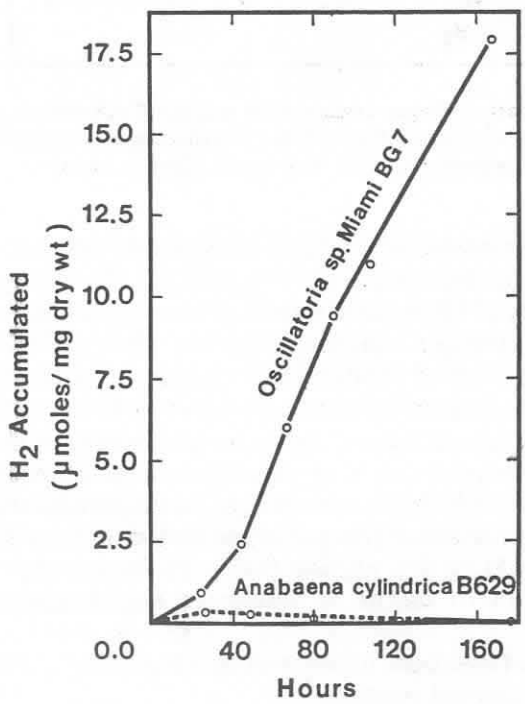


Fig. 10. Comparison of long-term hydrogen photoproduction and accumulation in closed vessels between Oscillatoria sp Miami BG 7 and Anabaena cylindrica B 629. (Reddy, Kumazawa and Mitsui, unpublished)

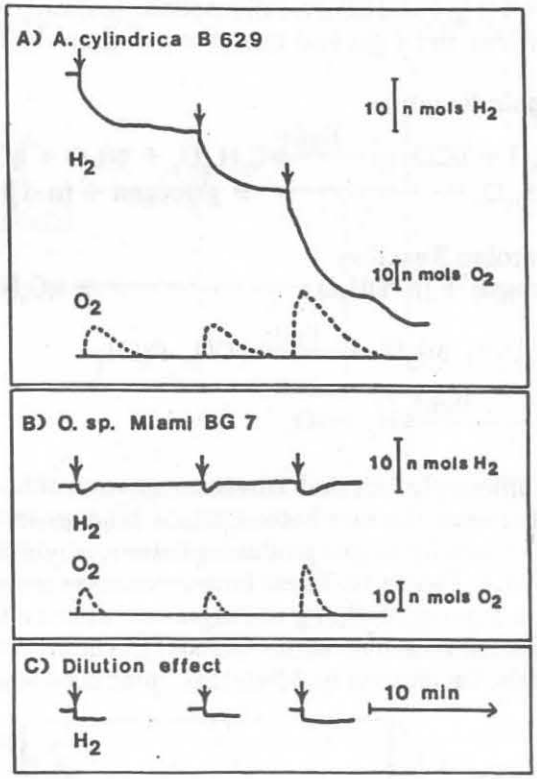


Fig. 11. Comparison of dark hydrogen consumption activity (Oxyhydrogen reaction) between marine *Oscillatoria* sp. Miami BG 7 and *anabaena cylindrica* B 629 using hydrogen-Oxygen electrodes. Identical amounts of blue-green algae were present in both blue-green algae suspensions (Kumazawa and Mitsui, unpublished)

gen in large quantities for several days. Figure 11 shows the comparison of hte dark uptake hydrogenase (hydrogen consumption) activity between the two species using hydrogen and oxygen electrodes and identical amounts of blue-green algae. High rates of uptake hudrogenase activity (hydrogen consumption) was observed in *Anabaena cylindrica* whereas no activity was detected in Miami BG 7. Sustained long-term hydrogen productin was observed over a wide range of light intensities from 20 to 1000 μ Einsteins/$m^2$/sec (Fig. 12). This means that maximum rates of hydrogen photoproduction can be maintained at light intensities less than 1/10 that is required to saturate photosynthesis, while at the same time there is no photoinhibition at high light intensities. Miami BG 7 exhibited high rates of hydrogen photoproduction over prolonged periods of time. The maximum rate observed so far is 300 ml $H_2$/g dry wt/day (Table 3). Recent results indicate hydrogen photoproductin values of 1.1 ml/ml of blue-green algal suspension/day. (Phlips and Mitsui, unpublished).

In general, growth and hydrogen production by Miami BG 7 is tolerant to a wide range of physical and environmental conditions[55].

**One-step Simultaneous Hydrogen and Oxygen Photoproduction Using Aerobic Nitrogen-fixed Unicellular Marine Blue-green Algae and Salt Water as a Hydrogen Donor**

Several strains of aerobic nitrogen-fixing unicellular (single-cell) blue-green algae, *Sy-*

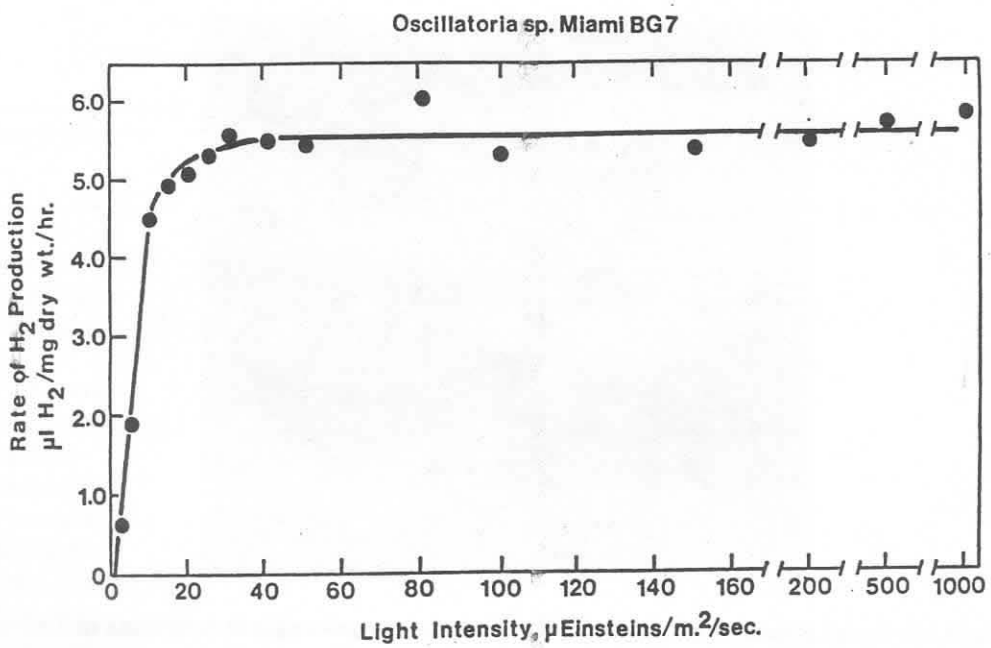


Fig. 12. Dependence on light intensity of hydrogen production in marine *Oscillatoria* sp. Miami BG 7[55].

*nechococcus spp*, were isolated from the sub-tropical marine environment[30,34] (Fig. 13) These strains grew exceptionally well (e.g doubling times, approximately 20 hrs.) even under vigorous aeration without any combined nitrogen sources such as nitrate and ammonia[9,10]. Molecular nitrogen grown cells showed very high aerobic nitrogenase activity[10]. Since nitrogenase is known to be extremely sensitive to oxygen and since oxygen is produced photosynthetically inside cells during growth, these strains probably have a unique mechanism to protect nitrogenase from the inhibitory effect of oxygen.

When cells from early log phase were incubated in a closed vessel, hydrogen and oxygen were evolved simultaneously for 5 days at a molar ratio of 2:1 under continuous light (Fig. 14). It is well-known that in green algae (suchas *Anabaena cylindrica)* simultaneous hydrogen and oxygen production can last only for a very short period (e.g. minutes to hours) in closed vessels, unless special treatments such as inert gas sparging[76] by continuous flow

TABLE III

$H_2$ Photoproduction Activity by Immobilized Cells of Marine *Oscillatoria* sp. Miami BG 7 (Phlips and Mitsui, unpublished)

| Incubation | $H_2$ Photoproduced (ml/g Cell Dry Wt/Day) |
|---|---|
| 1st day | — |
| 2nd day | 302 |
| 3rd day | 288 |
| 4th day | 228 |
| 5th day | 216 |

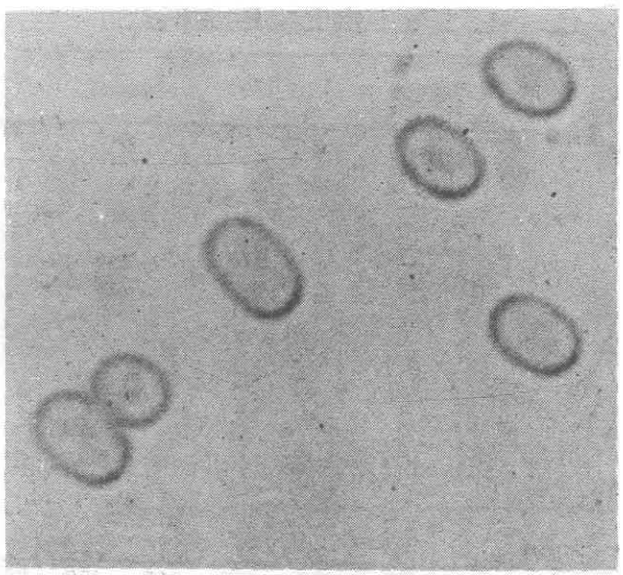

Fig 13. Microscopic picture of a marine aerobic nitrogen fixing blue-green algae, *Synechococcus* sp. (León and Mitsui, unpublished)

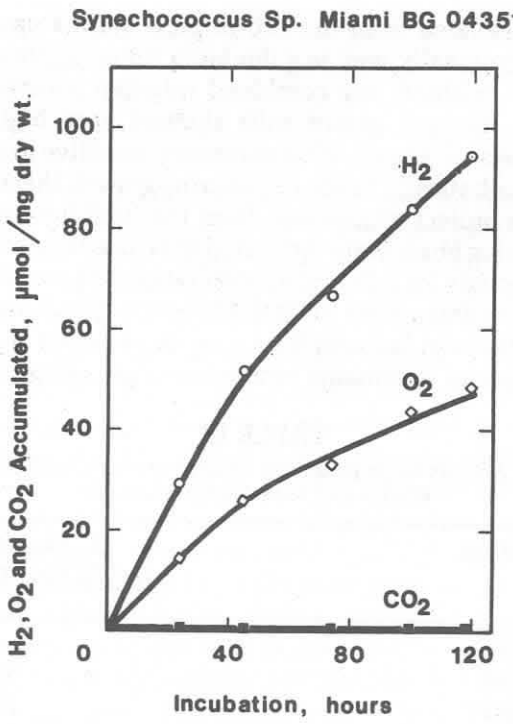


Fig. 14. Prolonged simultaneous, stoichiometric hydrogen and oxygen production from water by unicellular, nitrogen-fixing blue-green alga, *Synechococcus* sp. Miami BG 043511 (Reddy and Mitsui, unpublished)

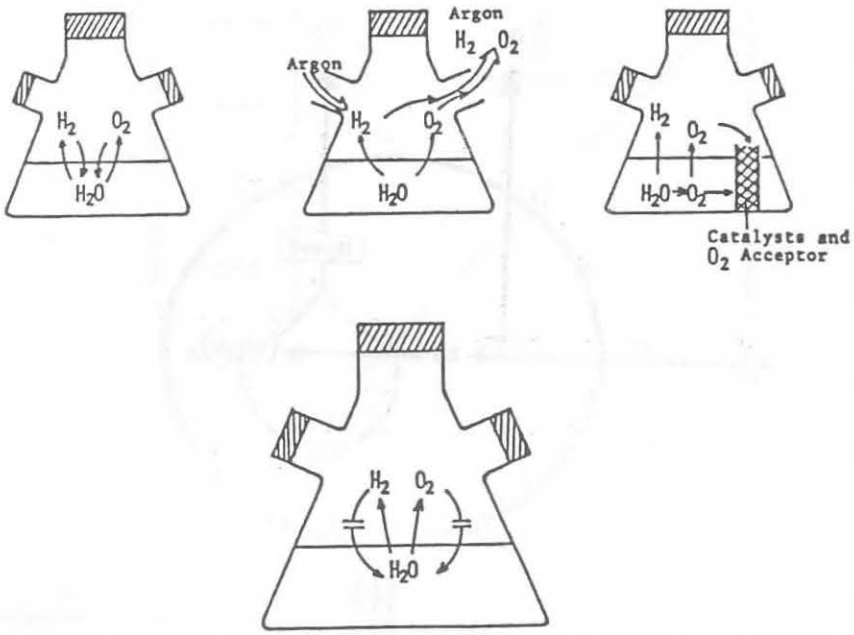


Fig. 15. Comparison of continuous hydrogen photoproduction systems (see text for details)

through system to remove accumulated hydrogen and oxygen and/or applying chemicals to trap oxygen[57] are used. Figure 15 illustrates flowthrough and oxygen trapping systems in a closed vessel. Prolongd hydrogen and oxygen evolution for very long periods of time in a closed vessel, is one of the unique characteristics of these nitrogen fixing *Synechococcus* strains. During this process there was no net change in cellular carbohydrate content (Reddy and Mitsui, unpublished). This evidence together with the observations of 1) stoichiometric hydrogen and oxygen production at a molar ratio of 2.0 and 2) no carbon dioxide production, indicate that water is the ultimate source of hydrogen, as illustrated in Figure 16.

One of the aerobic nitrogen-fixing unicells, *Synechococcus* sp. Miami BG 043511, was studied for the optimization of hydrogen production. (Reddy and Mitsui, unpublished). The results of this study indicate that, 1) Hydrogen photoproduction differs with culture age; early log phase cells exhibited the highest rates of hydrogen production. 2) Removal of external $CO_2$ and $N_2$ from the gas phase enhanced hydrogen production significantly, as these gases were found to be competitive inhibitors of hydrogen production. 3) Light saturated conditions were essential for stoichiometric hydrogen and oxygen production from water. Under light limited conditions the hydrogen photoproduction reaction was inhibited with the eventual release of $CO_2$[58] In another experiment (Reddy and Mitsui, unpublished) it was shown that BG 043511 simultaneously photoproduced hydrogen and oxygen for several days at high rates (430 ml $H_2$/g dry wt/day). A 5 ml algal suspension produced 3 ml $H_2$ in a one-day incubation period. Simultaneous hydrogen and oxygen production lasted until 24% $H_2$ and 12% $O_2$ was built up in the closed vessel. Under optimal conditions, the rate of hydrogen production recorded was as high as 2 µmoles/mg dry wt/hr or 250 µmoles/mg Chl/hr.

These data indicate that the aerobic nitrogen-fixing unicellular blue-green alga *Synechococcus* sp. Miami BG 043511 as well as the previously well studied non heterocystous

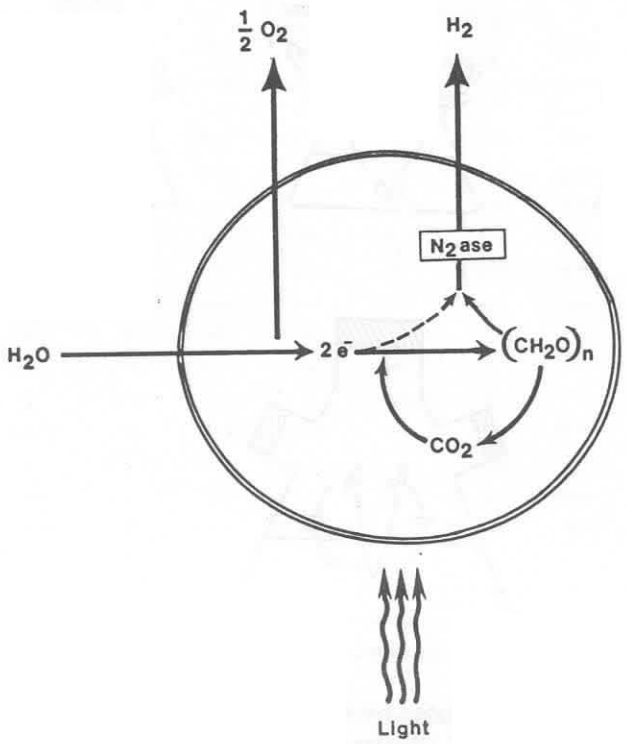


Fig. 16. Scheme for one-step stiochiometric hydrogen and oxygen photoproduction from water by unicellular nitrogen fixing blue-green alga *Synechococcus* sp. Miami BG 043511 (Ref. 41 and (Reddy and Mitsui, unpublished)

blue-green alga *Oscillatoria* sp. Miami BG 7, are high and stable hydrogen producing blue-green algae. Efforts are underway to further prolong the period of continuous hydrogen production in both cell free and immobilized cell systems of these strains. Apparently this strain can use exogenous organic as electron donors for hydrogen production (Fig. 17).Later it was found that the same thing is true for the filamentous species Miami BG7 (Fig. 18). This opens up the possibility of using waste water, rich in organics, to fuel biological hydrogen production by blue-green algae. However, photosynthetic bacteria in general show much higher rates of hydrogen production using organic substances.

**Hydrogen Production by Photosynthetic Bacteria**

Like blue-green algae, photosynthetic bacteria are capable of using the sun's energy to grow, fix nitrogen and produce hydrogen gas. However, the process of photosynthesis in this group is a one-photosystem reaction requiring an electron donor more reduced than water[42]. In our survey of hydrogen producing organisms, we concentrated our attentin to two major types of photosynthetic bacteria, sulphur and non-sulphur strains. We found several species in both groups which exhibited exceptional growth rates and hydrogen production capacity.

Photosynthetic sulphur bacteria (Chromatiaceae and Chlorobiaceae) have the unique ability to utilize sulphide as an electron donor for growth and hydrogen production. Sulphide is one of the major pollutants in the environment and can be found in sewage, industrial and agricultural waste, polluted river, marine water and sediments. Therefore, hydro-

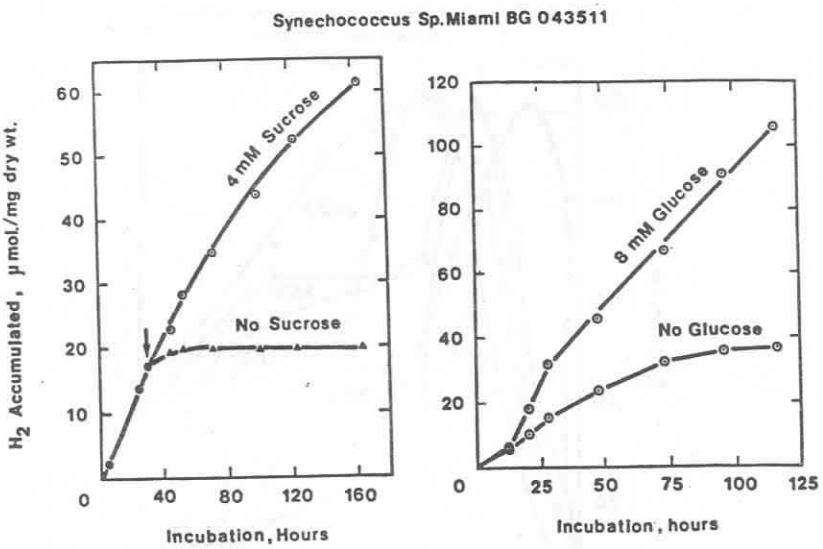


Fig. 17. Effect of externally added sucrose and glucose on hydrogen production in unicellular nitrogen fixing blue-green alga *Synechococcus* sp. Miami BG 043511. (Reddy and Mitsui, unpublished). Rates of hydrogen production do not reflect optimum coditions.

gen production systems using photosynthetic sulphur bacteria coupled with removal of sulphide from the environment and waste could have significant benefits[25.41].

Numerous strains of photosynthetic sulphur bacteria were isolated from tropical and sub-tropical environments including marine water, marine sediments, salt marsh, salt pro-

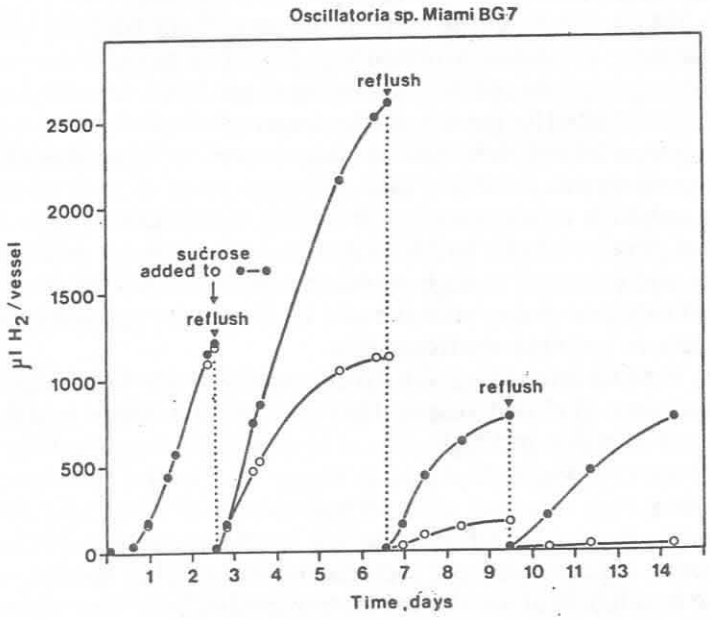


Fig. 18. Effect of externally added sucrose on prolonged hydrogen production in marine *Oscillatoria* sp. Miami BG 7 (Phlips and Mitsui, unpublished). Rates of hydrogen production do not reflect optimum conditions.

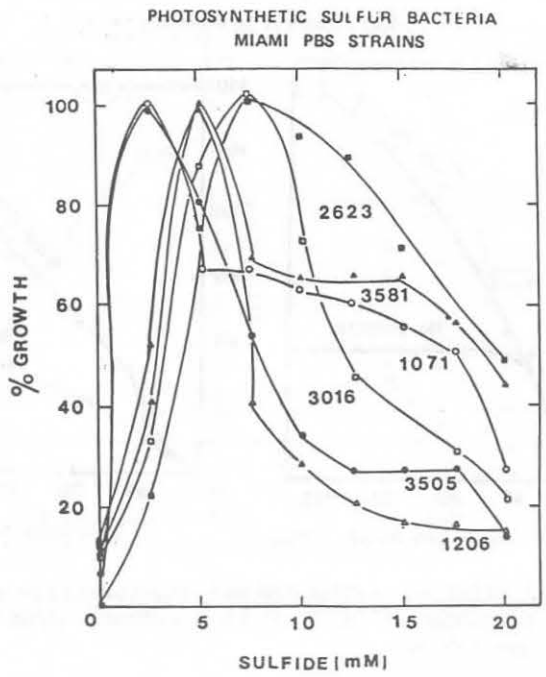


Fig. 19. Growth of various strains of marine photosynethetic bacteria as affected by sulphide concentration (Renuka, Ikemoto and Mitsui, unpublished)

duction fields, living and decaying sea grasses, mangrove leaves, seaweeds etc.[28,29,30,35,38] Many of these strains exhibited hydrogen photoproduction[29,38,42].

Among the 225 strains of marine sulphur photosynthetic bacteria tested in the collection of this laboratory, 60 strains exhibited high growth rates and biomass yields in culture medium containing sulphide and malate. Among these, 53 strains with high hydrogen production ability were tested for growth and hydrogen photoproduction at sulphide concentrations ranging from 2.5 to 20mM. Most strains showed optimum growth at 2.5 to 7.5 mM sulphide, but some strains exhibited high optimum range of 10 to 20 mM sulphide (Fig. 19). Optimum sulphide concentrations for hydrogen production varied widely[21].

The fact that photosynthetic bacterial strains have different prefrences for sulphide concentrations and tolerance to high sulphide concentrations for growth and hydrogen production, indicate that proper strains could be chosen for application according to sulphide abundance in polluted environments.

Most strains tested exhibited uptake hydrogenase activity which diminishes the accumulation of hydrogen in closed vessels. However, several strains whcih apparently lack this activity showed stable and high rates of hydrogen photoproduction, as in the case of strains Miami PBS 3581 and 2623 shown in Figure 20. The fact that many of these strains take up sulphide at high rates and use it for both growth (fig. 21) and hydrogen production (fig. 22) makes them prime candidates for use in treatment of sulphide waste.

The other group of photosynthetic bacteria, the non-sulphur species, do not exhibit the great tolerance to sulphide shown by the former species, but do have the ability to utilize a remarkably broad range of organic substrates for growth (Table 4) and hydrogen production (Table 5). In terms of rates of hydrogen production, some of the strains exhibited high-

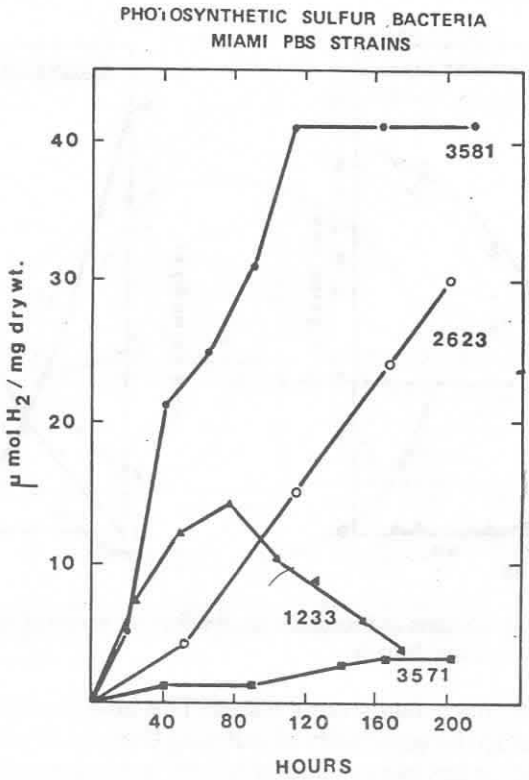


Fig. 20. Time courses of hydrogen photoproduction by four strains of marine photosynthetic sulphur bacteria (Ikemoto, Renuka and Mitsui, unpublished)

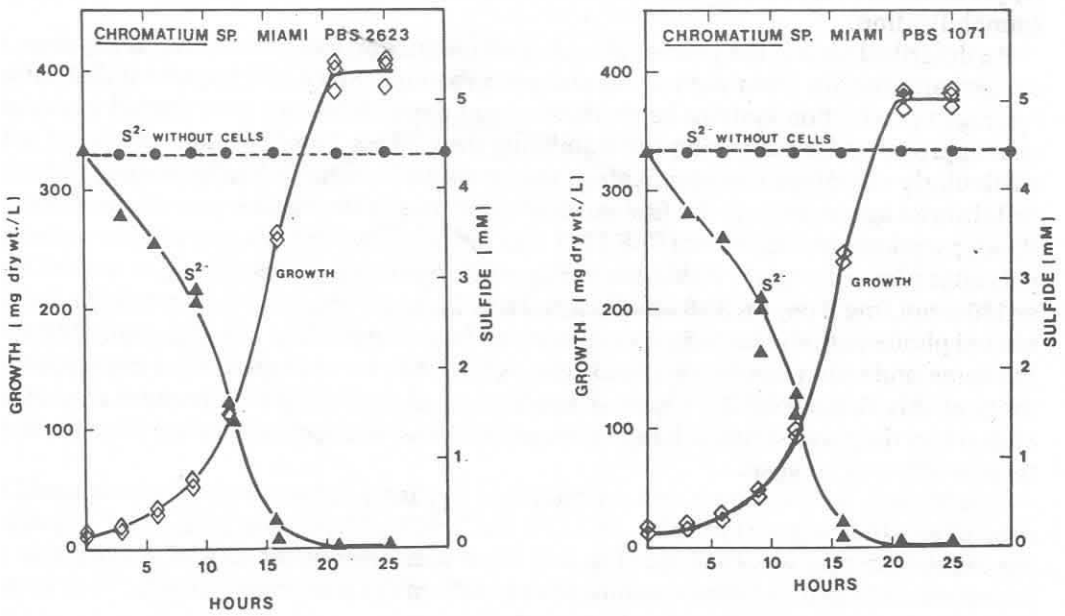


Fig. 21. Growth and sulphide removal by two strains of marine photosynthetic sulphur bacteria (Renuka and Mitsui, unpublished)

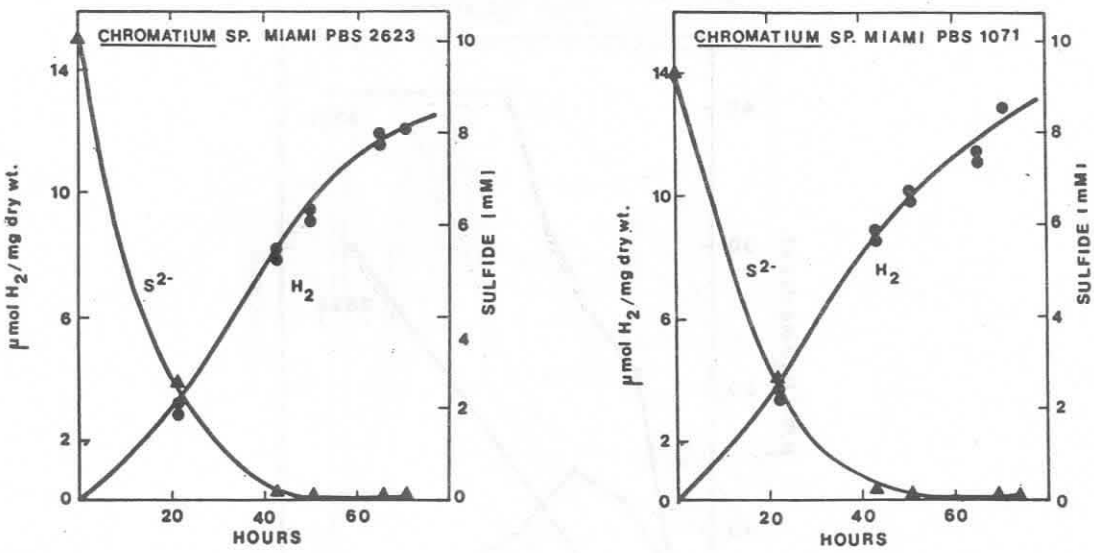


Fig. 22. Hydrogen photoproduction and sulphide removal by two strains of marine photosynthetic sulphur bacteria (Renuka and Mitsui, unpublished)

er rates than any other microbial strains tested. The broad substrate specificity of these bacteria clearly opens up the possibility of utilizing them in the treatment of complex organic waste. This concept has been tested in Japan[22] but not in conjunction with concomitant production of hydrogen gas.

**Approaches for Enhancing and Stabilizing Hydrogen Production by Immobilization.**

As described above, the process of hydrogen production can be sensitive to exposure to oxygen and certain other environmental perturbations. Since it is important that future hydrogen production systems be productive and dependable, we have started to explore new approaches for enhancing and stabilizing the process. One technique we found to be particularly effective in accomplishing these goals was to immobilize hydrogen producing cells into an agar matrix. In the first series of experiments the marine non-sulphur bacteria *Rhodopseudomonas* sp. Miami PBE 2271 was immobilized on transparent agarose-coated polyester film with agar[26]. As shown in Figure 23, hydrogen was produced at rates as high as 180 μmol/mg B ch/hr, 3.96 μmol/mg protein/hr, and 1.98 μmol/mg dry cell/hr. Immobilized photosynthetic bacteria were protected from oxygen (Fig. 24). The strain PBE 2271 has some protection mechanism to oxygen, which may be attributed to the respiration activity of this strain. PBE 2271 further established an anaerobic environment around the cells when they were immobilized in the gel matrices, thus protecing them from the deleterious effect of oxygen.

In addition, it was shown that immobilized marine photosynthetic bacteria could be used over wide ranges of salinity. At 100% NaCl, 50% of hydrogen production activity of the maximum rate was retained (Fig. 25). Even with freshwater 93% of activity was retained whereas cell suspensions showed only 52% of the maximum activity. Thus immobilized cells were also protected from osmotic stress.

As previously discussed, utilization of organic wastes as an electron donor is one of the most important factors in application of hydrogen photoproduction. Aqueous suspensions

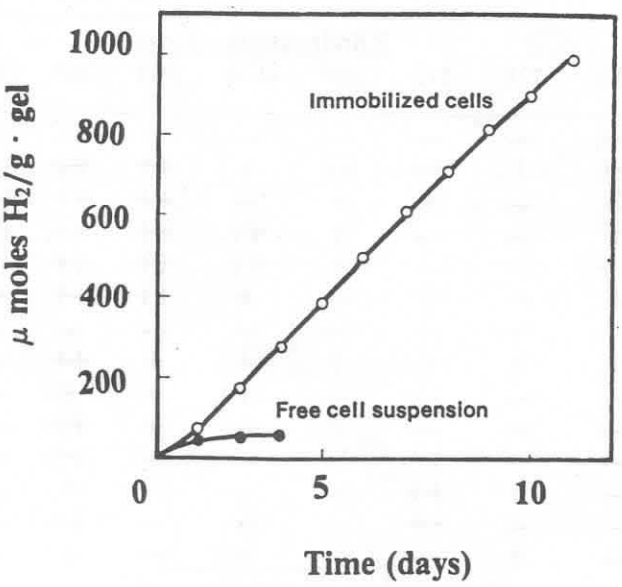


Fig. 23. Prolonged hydrogen photoproduction by immobilized marine photosynthetic non-sulphur bacterium, *Rhodopseudomonas* sp. Miami PBE 2271[26].

of photosynthetic bacteria can utilize certain organic wastes to produce hydrogen. However, it is difficult to separate photosynthetic bacteria after use for hydrogen production. Immobilized cells can be easily separated from wastewater. Therefore, immobilized cells are suitable for organic waste and wastewater treatment. Reduction of the total organic carbon (TOC) and biological oxygen demand (BOD) of wastewater was observed when immobilized cells were employed for wastewater treatment[41]. Moreover, this system is based on seawater. This system is applicable to pollution control in marine environments which have various salinites.

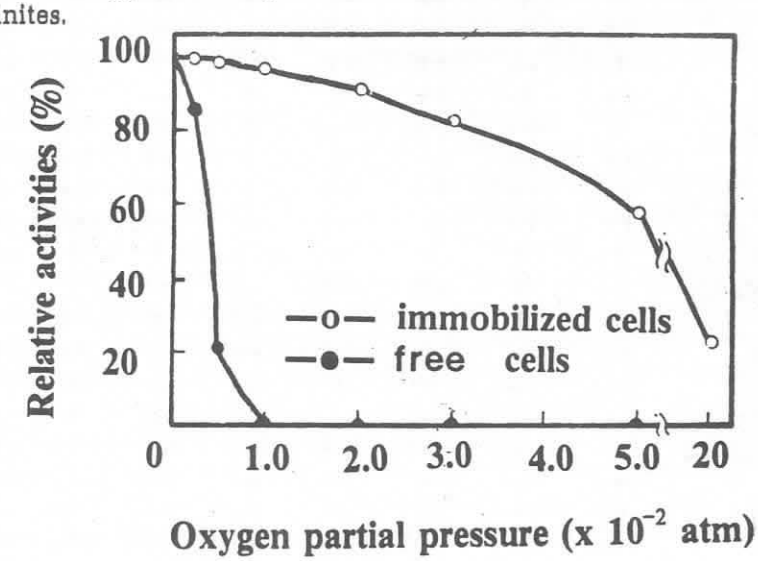


Fig. 24. Effect of oxygen partial pressure on hydrogen production in free and immobilized cells of marine photosynthetic non-sulphur bacterium *Rhodopseudomonas* sp. Miami PBE 2271[26].

## TABLE IV

Growth Substrate Specificity of Marine Rhodospirillaceae
(Matsunaga and Mitusi, Unpublished)

| Substrates | Rhodopseudomonas sp. 115 | 136 | 217 | 227 | 258 | 263 | 266 | 285 | 301 | 338 |
|---|---|---|---|---|---|---|---|---|---|---|
| Formate | ++ | — | — | — | — | + | — | — | — | ++ |
| Acetate | ++ | + | ++ | ++ | + | ++ | ++ | ++ | — | + |
| Propionate | — | — | + | + | — | ++ | ++ | ++ | — | + |
| Butyrate | + | — | — | + | ++ | ++ | ++ | ++ | ++ | ++ |
| Valerate | ++ | + | — | + | ++ | ++ | ++ | ++ | + | ++ |
| Caproate | + | — | + | — | + | ++ | ++ | + | + | ++ |
| Caprylate | — | — | — | — | — | — | — | — | — | — |
| Pyruvate | + | + | + | + | + | + | ++ | — | + | ++ |
| Lactate | — | — | — | — | + | + | + | — | — | — |
| Citrate | — | — | — | + | — | + | ++ | — | — | ++ |
| Malate | — | — | ++ | ++ | — | — | ++ | — | — | + |
| Fumarate | — | + | ++ | — | — | — | + | — | — | + |
| Succinate | — | — | ++ | — | — | — | ++ | — | — | + |
| Tartrate | — | + | — | — | + | — | + | + | — | — |
| B-Glycero-phosphate | + | + | — | — | + | — | + | — | — | + |
| Malonate | + | — | + | + | — | — | + | + | — | ++ |
| Methanol | + | — | + | + | — | + | + | + | — | + |
| Ethanol | — | — | ++ | + | — | + | + | + | + | + |
| Glycerol | — | — | + | — | — | — | ++ | + | + | + |
| Arabinose | + | — | + | — | — | — | + | ++ | + | + |
| Dextrin | ++ | + | ++ | ++ | + | + | + | ++ | ++ | ++ |
| Glucose | ++ | + | ++ | ++ | ++ | + | ++ | ++ | ++ | + |
| Fructose | + | + | ++ | ++ | + | — | ++ | ++ | ++ | + |
| Lactose | + | + | ++ | — | + | + | + | + | + | + |
| Maltose | ++ | + | ++ | ++ | + | + | + | + | ++ | ++ |
| Mannitol | + | — | ++ | ++ | ++ | + | ++ | ++ | + | ++ |
| Xylose | ++ | + | ++ | + | + | — | + | — | + | ++ |
| Cellobiose | + | ++ | ++ | ++ | + | ++ | ++ | ++ | ++ | ++ |
| Mannose | ++ | ++ | + | + | + | ++ | ++ | ++ | + | ++ |
| Sorbitol | ++ | ++ | ++ | ++ | + | ++ | ++ | + | + | + |
| Sucrose | ++ | ++ | + | ++ | ++ | ++ | ++ | ++ | + | ++ |
| Cellulose | + | + | + | + | — | + | + | — | — | + |
| Soluble Starch | + | + | ++ | ++ | — | ++ | + | ++ | + | — |
| Pectin | + | + | ++ | ++ | ++ | + | ++ | ++ | + | ++ |
| Aspartate | — | — | + | ++ | — | — | — | + | + | — |
| Arginine | — | — | + | + | — | — | — | + | — | — |
| Glutamate | — | — | — | — | — | — | + | — | — | — |
| CasaminoAcid | ++ | +++ | +++ | ++ | +++ | ++ | +++ | +++ | + | ++ |
| Phenol | — | — | — | — | — | — | — | — | — | — |
| Phthalate | — | — | — | — | — | — | + | — | — | — |
| Benzoate | — | — | — | — | — | — | + | — | — | — |
| Thiosulphate | ++ | ++ | ++ | ++ | + | ++ | ++ | + | ++ | + |

Symbols:
—, growth not better than in control without added carbon source
+, light growth ($OD_{650}$ up to 0.1)
++, moderate growth ($OD_{650}$ 0.1 to 0.5)
+++, heavy growth ($OD_{650}$ more than 0.5)

TABLE V

Hydrogen Production from Various Substrates by Marine *Rdodopseudomonas* sp. Miami PBE 2271 (Mastuaga and Mitsui, Unpublished)

| Substrate | Hydrogen productivity (μ moles/mg protein/hr) |
|---|---|
| Formate | — |
| Acetate | 4.9 |
| Propionate | — |
| Butyrate | 1.5 |
| Citrate | — |
| Fumarate | 6.9 |
| B-Glycerophosphate | 0.9 |
| Lactate | 3.4 |
| Malate | 8.0 |
| Pyruvate | 5.1 |
| Succinate | 4.5 |
| Tartarate | 0 |
| Valerate | 0 |
| Caproate | 0 |
| Caprylate | 0 |
| Malonate | 0.8 |
| Methanol | 0 |
| Ethanol | 0 |
| Glycerol | 0 |
| Arabinose | 0.2 |
| Dextrin | 5.1 |
| Glcose | 5.3 |
| Fructose | 4.8 |
| Lactose | 0 |
| Maltose | 5.7 |
| Mannitol | 4.8 |
| Xylose | 2.7 |
| Cellobiose | 0.2 |
| Mannose | 0 |
| Sorbitol | 0.2 |
| Sucrose | 4.5 |
| Cellulose | 2.3 |
| Pectin | 1.4 |
| Asparate | 0 |
| Arginine | 0 |
| Glutamate | 0 |
| Casamino Acid | 0.2 |
| Phenol | 0 |
| Phthalate | 0 |
| Benzoate | 0 |

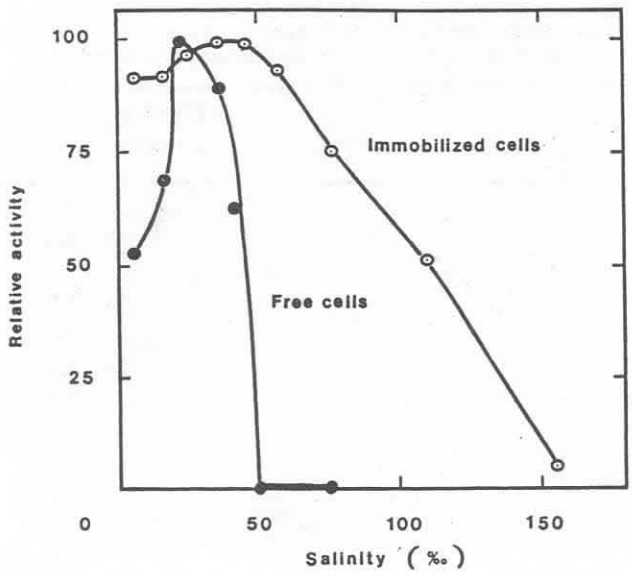


Fig. 25. Effect of salinity on hydrogen production in free and immobilized cells of marine photosynthetic non-sulphur bacterium *Rhodopseudomonas* sp. Miami PBE 2271[28].

In another set of experiment the marine photosynthetic sulphur bacterium, Chromatium sp. Miami PBS 1071 was tested in immobilization.[20] Whole cells were immobilized in an agar gel matrix and grown on either molecular nitrogen or ammonia as the nitrogen source. Immobilized cells grew in the agar gel matrix but not in the surrounding suspension media. High biomass yield was obtained. Hydrogen photoproduction by immobilized cells was carried out by periodic addition of supplement sulphide in the reaction system (Fig. 26). Sulphide was the sole electron donor used for the reaction. $CO_2$ was not producted and only $H_2$ was evolved in this system. Hydrogen gas in this immobilized system was continuously produced for several weeks at a constant rate. This is in contrat to free cell systems where the hydrogen production dropped within a few days. The rates of hydrogen photoproduction, on a reaction volume basis, were improved by growing cells in an immobilized system. Immobilized ammonia grown cells produced hydrogen at the same rates as $N_2$ grown cells, but a short lag period was observed before initiation of production. Rates of hydrogen production up to 6 μmoles/g gel/hr were observed. In comparisons of hydrogen production in immobilized and free-cell states the former exhibited far superior yield for two species tested (Fig. 27). These results indicate that immobilized systems using photosynthetic bacteria have advantages over the free cell system including: 1) cells can grow to high biomass yield in immobilized gel. Thus, all harvesting procedures (e.g. centrifugation) are eliminated and hydrogen production can be carried out in the same reactor, 2) continuous hudrogen production can be carried out without the loss of cells, and 3) hydrogen production activity of the cells is stabilized for longer periods of time.

Based on the successful application of this technique to photosynthetic bacteria we applied the method to the blue-green alga Miami BG 7. (Phlips and Mitsui, unpublished). Blue-green algal cells immobilized in agar exhibited hydrogen production for more extended periods of time (Fig. 28) than free cells, thereby enhancing the ultimate yield of hydrogen gas (Table 3). Immobiization also improved the response of cells to oxygen. Similar-

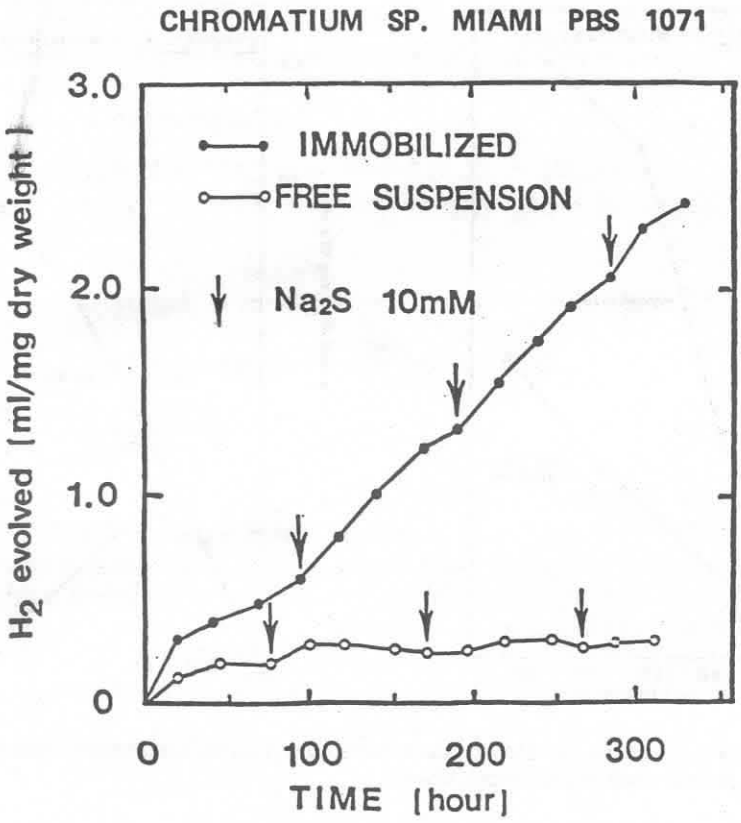


Fig. 26. Effect of frequent addition of sulphide on prolonged hydrogen photoproduction in free and immobilized cells of marine photosynthetic sulphur bacterium *Chromatium* sp. Miami PBS 1071[20].

ly, to the photosynthetic bacterial systems[20,26,41] immobilization offered ease of handling, prevention of loss of cells and elimination of harvesting procedures. These findingds indicate that the recycling biological hydrogen photoproduction system using immobilized cells or marine blue-green alga *Oscillatora* sp, Miami BG7 and *Synthococcus* sp Miami BG 043511 has promising potential in applied bioenergy system developments.

**Outdoor Hydrogen Production**

The next step in achieving the goal of a viable hydrogen production technology will be to test and implement actual systems for hydrogen production in the natural environment. As a first step toward realizing this goal, a study was made of the response of hydrogen photoproduction in the blue-green alga *Oscillatoria* sp. Miami BG7 to natural fluctuations in sunlight.[56] The primary objectives of this work were to establish whether 1) high rates of hydrogen production could be maintained within the context of a diurnal light cycle and 2) if high midday light intensity would inhibit hydrogen production. The five litre bioreactors used in these tests were specially designed to receive sunlight in a manner similar to flat plate solar collectors (Fig. 29). Cells for the reactors were grown in 250 litre algal culture cylinders (Fig. 29). Temperature and the circulation rate within the algal suspensions were maintained constant. Light intensity was adjusted by using neutral density material. It was found that the ability of cells to evolve hydrogen increased dramatically

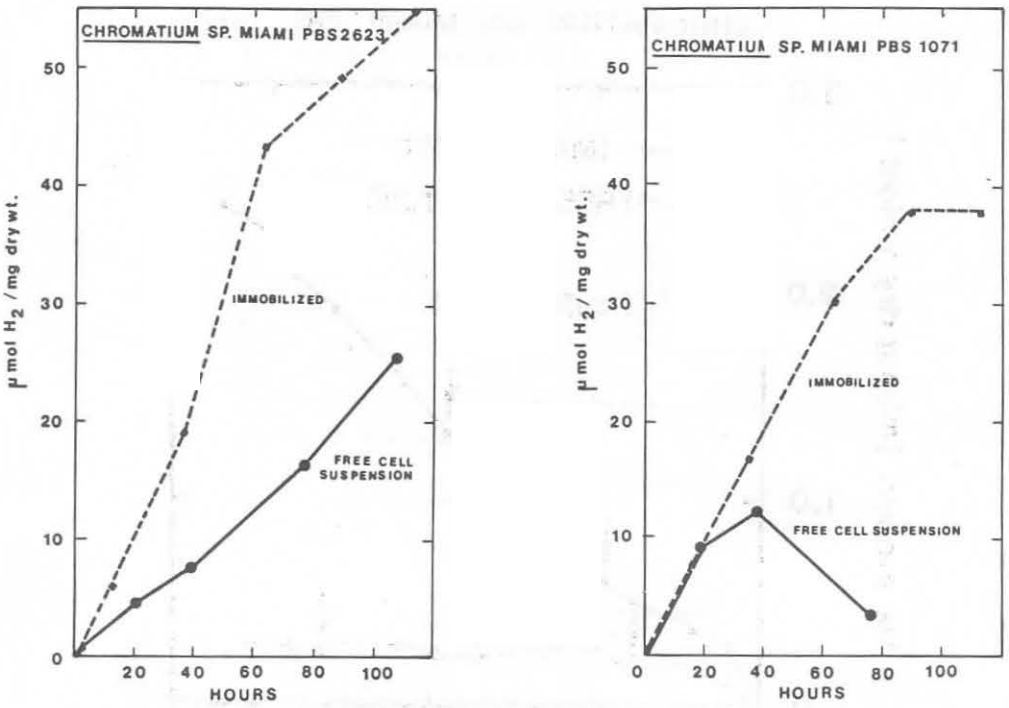


Fig. 27. Comparison of hydrogen photoproduction by two strains of marine *Chromatium* under free and immobilized conditions (Ikemoto and Mitsui, unpublished).

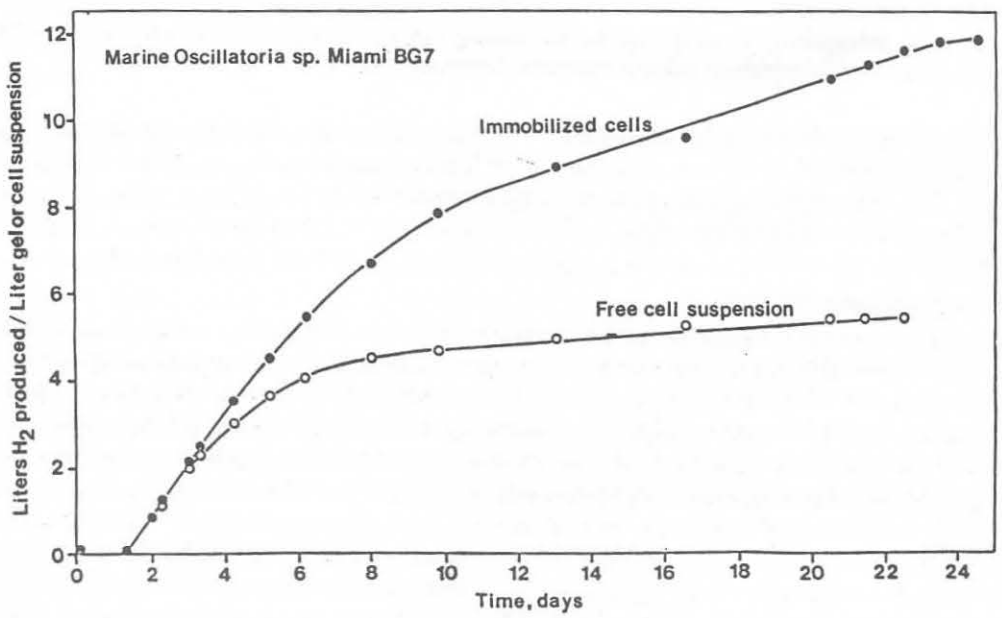
Marine Oscillatoria sp. Miami BG7
Immobilized cells
Free cell suspension
Liters H2 produced / Liter gel or cell suspension
Time, days

Fig. 28. Long term hydrogen production by free and immobilized cells of marine *Oscillatoria* sp. Miami BG7 (Phlips and Mitsui, unpublished)

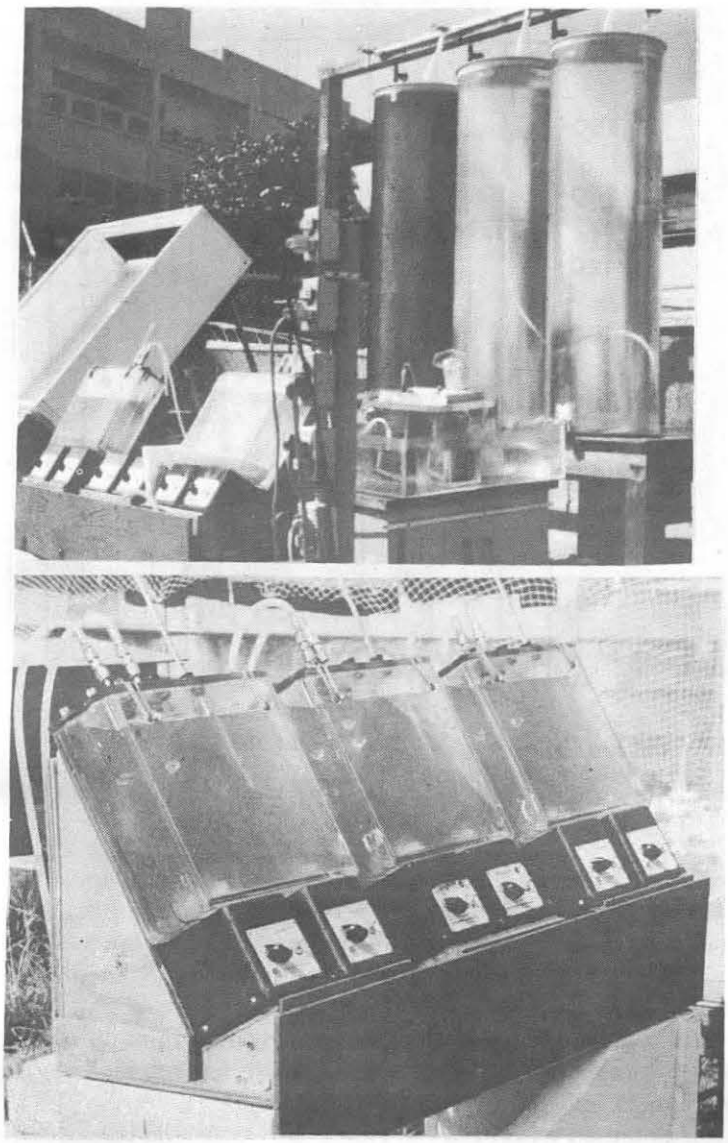

Fig. 29. Blue-green culture and bioreactor system placed outdoors for hydrogen production. These reactors contain marine blue-green alga *Oscillatoria* sp. Miami BG 7 (Phlips and Mitsui, unpublished).

during the first two light periods, and peaked in the third day (Fig. 30) (Phlips and Mitsui, unpublished). High productivity was observed for over a week. No hydrogen production production or uptake was observed at night. The maximum rates of hydrogen production observed were similar to those obtained in the previous laboratory experiments, approximately 8.2 $\mu$l $H_2$/mg dry wt./hr. Full midday sunlight intensities (i.e. 2000 uEinsteins/$m^2$/sec.$^2$) were found to be somewhat inhibitory to hydrogen production, particularly after numerous days of exposure. The magnitude of this inhibition was dependent on a number of factors, including cell age. Cells for these experiments were cultured on a limited amount of ammonium as the nitrogen nutrient source. Optimal rates of hydrogen produc-

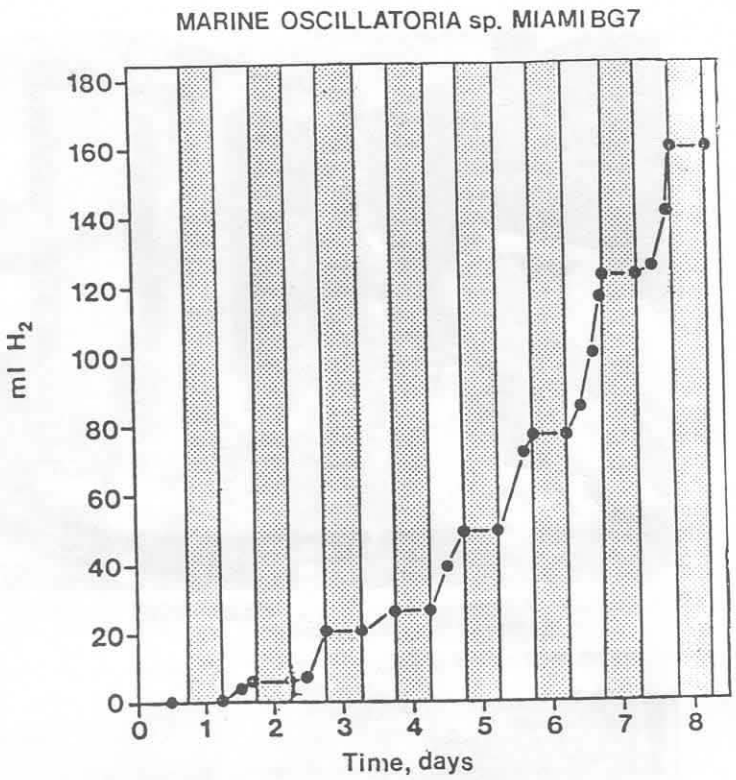


Fig. 30. Outdoor hydrogen production by 350 ml of immobilized gel, containing *Oscillatoria sp. Miami BG 7* (Phlips and Mitsui, unpublished)

tion were observed after cultures became depleted of nitrogenous nutrients. This depletion process was accompanied by a progressive reduction in the cellular content of light harvesting pigment. This coincided with an increasing resistance of hydrogen production to high light intensity. Outdoor hydrogen production by immobilized cells of Miami BG 7 are shown in Figure 30. Stable hydrogen production was observed for a week.

Outdoor experiments were also performed with the immobilized photosynthetic sulphur bacteria Miami PBS 1071. Hydrogen production responded very well to the outdoor conditions (Figs. 31 and 32).

The results obtained with both the blue-green algae and photosynthetic bacteria indicate that hydrogen production systems are adaptable to outdoor conditions. Complete view of outdoor fish-algal culture and algal and bacterial hydrogen photoproduction research which are successfully conducted in natural sunlight conditions are shown in Figure 33.

## THE DEVELOPMENT AND UTILIZATION OF CHEMICAL PRODUCTS OF ALGAE AND PHOTOSYNTHETIC BACTERIA

There is an enormous range of chemical substances produced by algae and photosynthetic bacteria which are of interest to man. These substances have been surveyed in freshwater algae for many years; however, the survey of these substances from marine algae, particularly the marine microalgae, have not yet been made extensively. New strains iso-

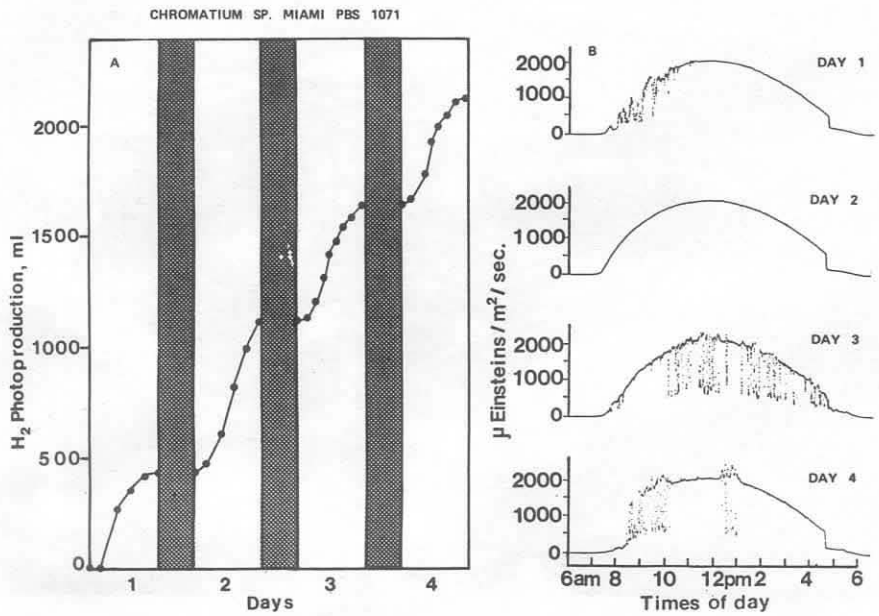


*Fig. 31. Outdoor hydrogen production by immobilized marine photosynthetic sulphur bacterium Chromatium* sp. Miami PBS 1071. Figure 31B shows the light intensities during the 4 day incubation period (Ikemoto and Mitsui, unpublished)

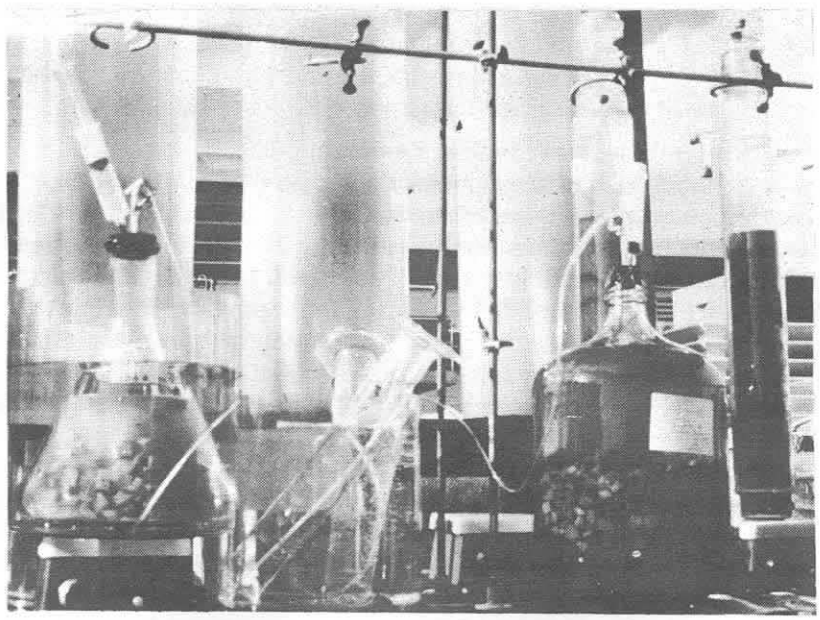

Fig. 32. Various types of bioreactors placed outdoors for photosythetic bacterial hydrogen production (Ikemoto and Mitsui, unpublished).

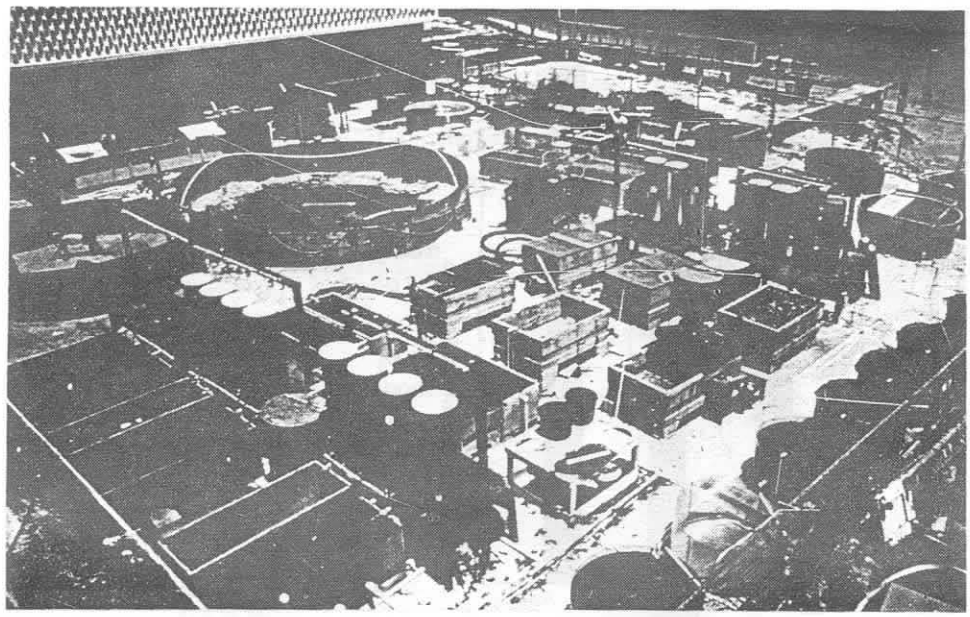

Fig. 33. Bird's-eye view of overall outdoor fish-algal culture and hydrogen photoproduction experimental facilities. Lower right corner; shows fish-nitrogen fixing marine blue-green algal culture system. Left corner; fish-macroalgal coculture system. Left top right corner; blue-green algal and photosynthetic bacterial hydrogen photoproduction systems

lated from the marine environment should have a high potential for the discovery of new chemical products of use to man. We have oriented our research in two different directions, 1)investigation of biologically active sustances which may be useful in the drug and pharmaceutical industries and 2) examination of biochemical compounds which are produced in large quantities by blue-green algae and which may be utilized by the food, fertilizer, chemical or other consumer industries.

**Use of Marine Algae in Drug and Medical Research**

The survey of metabolically active substances produced by marine algae, and identification of those compounds, has received some attention in the past few years;[14,43] however, it remains a potentially profitable area for investigation. Several algae are already known to produce substances of medical importance. The anthelmintics, L-kimic acid and L-allo kimic acid have been obtained from the red-algae *Dignia simplex*;[44] domoic acid has been obtained from *Condria armata*.[65] Laminine, obtained from the brown-algae *Laminaria* sp. has been shown to be effective as an anti-hypertension agent.[52,66] In addition several algae are known to produce anti-bacterial compounds and cytotoxic compounds against human cancer cells.[14,61,64]

Blue-green algae occupy a unique taxonomic position among living organisms, between prokaryotic bacteria and eukaryotic plants. However, being prokaryotic, they are more closely related to bacteria than any other major classification of organisms. The cellular structure and biochemistry of blue-green algae differ significantly from those found in the other living forms. Although most blue-green algae do not produce toxic substances and can indeed be used for food and feed products,[13,41] some species produce substances which are highly toxic to higher and/or lower animal forms. Cytotoxic susbtances have been isolated from blue-green algae, *Oscillatoria* sp., *Schizothrix* sp. mixture, and *Tolypothrix* sp.[48,47]

Our research efforts in this area are focused on the hemolycines produced by blue-green algae. We isolated several algal strains producing hemolycines from an area of Biscayne Bay, Florida, in which a massive fish kill had occurred (Goodman and Mitsui, upublished). One of the isolated blue-green algae, *Synchococcus* sp., produced hemolycine only under certain environmental conditions (Reyes-Vasquez and Mitsui unpublished). Further chemcial study of one of these toxins showed that it contained a glycolipid group (Mitsui, Goodman, Kusumi, Reyes-Vasquez, Tanaka, Nomoto and Kodama, unpublished). Despite these findings our knowledge of the biochemistry of marine blue-green algal toxins is far inferior to our understanding of bacterial systems and freshwater algal systems.

Biologically active substances can be toxic to certain species, at the same time they can be beneficial as medicines, if administered in the right manner and at an appropriate dose. Medical researchers have long recognized the potential benefits of naturally provided substances. New biologically active substances may be found in many of the marine blue green-algae. Tropical coastal waters are an ideal region to undertake a search for new metabolically active substances. Our research efforts in this area were primarily centred on identifying strains of marine blue-green alage which exhibit significant activity in standard assays. Three main assays are being used at the present time; 1) anti-bacterial, anti-fungal activity, 2) neuro-muscular response of guinea-pig intenstine, and 3) insect mortality test (Kusumi and Mitsui, unpublished data). Various organic solvent extracts of cultured cells and medium were used for the screening of these assay systems. Out of 201 strains of marine blue-green algae tested so far, 12 have given a significant positive response in the first assay, 73 in the second assay and 26 strains in the third assay (Table 6). Nine algal extracts inhibited the growth of some grampositive bacteria and two inhibited the growth of some fungi. In the neuro-muscular response test, 49 strains showed contraction and 34 showed relaxation of muscle. This high frequency of neuro-muscular response activity was very interesting. We are now in the process of expanding this survey to include more species of blue-green algae and additonal bioassays. Isolation, purification and determination of these biologically active substances are also underway.

TABLE VI

Occurrence of Biological Active Substances in Marine Blue Green Algae (Kusumi and Mitsui, Unpublished Data)

| Marine Blue green algae Numbers of strains Genus | tested | Guinea-pig Intestine Biossay Contraction | Relaxation | Antibacterial and Antifungal activity | Insect mortality |
|---|---|---|---|---|---|
| *Nostoc* | 48 | 13 | 11 | 5 | 8 |
| *Anabaena* | 26 | 7 | 11 | 3 | 5 |
| *Calothrix* | 24 | 3 | 4 | 1 | 6 |
| *Synechococcus* & *Gloethece* | 30 | 11 | 5 | 2 | 1 |
| *Oscillatoria* & *Lyngbya* | 30 | 2 | 2 | 1 | 2 |
| Others | 43 | 3 | 1 | 0 | 4 |
| Total | 201 | 39 | 34 | 12 | 26 |

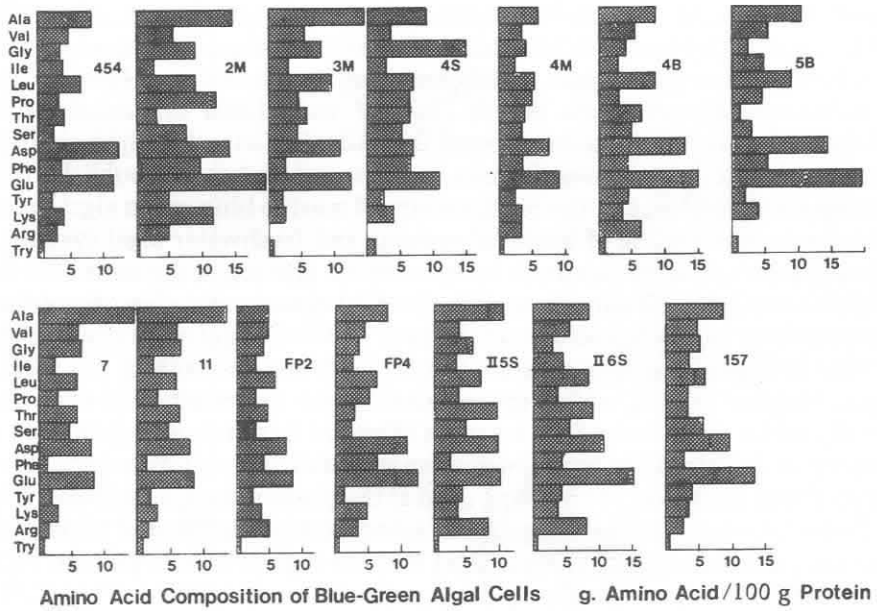


Fig. 34. Amino acid composition of some marine blue-green algae (Miyazawa and Mitsui, unpublished)

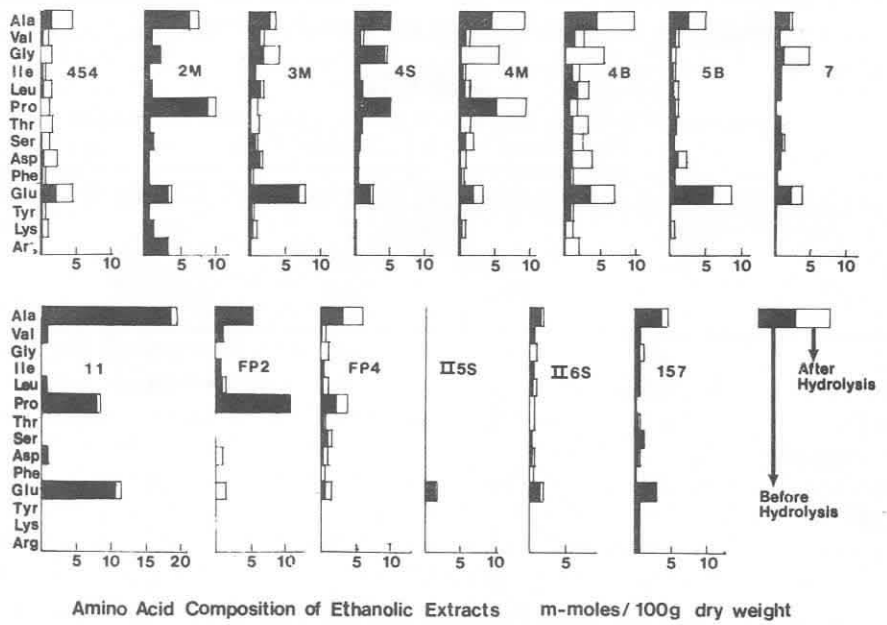


Fig. 35. Peptide composition of ethanol extracts of some marine blue-green algae (Miyazawa and Mitsui. unpublished)

### Use of Marine Algae in Food, Fertilizer and Chemical Production

There is a large number of chemcial substances produced by algae and marine plants which are of interest to man. In a project in which there is a potential for developing systems which will continuously produce large quantities of material, it is important to explore this resouce from a chemical point of view. Polysaccharides of algae, such as alginic acid from brown algae, carrageenans from red-algae and agar from red-algae are well known. These algal polysaccharides have been utilized previously, mainly in the food industry; however, the importance of the use of other algal polysaccharides has been recognized recently in the fields of medicine and chemistry. It was found that several marine blue-green algal strains collected in our laboratory produce a large quantity of extracellular polysaccharides. Some strains of marine blue-green algae in our collection also produce amino acids and peptides in different quantities as exemplified in Figures 34 and 35 (Miyazawa and Mitsui, unpublished). Algal by products such as vitamins, minerals, sterols, carotenoids, hydrocarbons, fatty acids and other chemical substances could also be utilized. There is some indication that our strains of blue-green algae and photosynthetic bacteria could produce quantities of some of these substances.

Blue-green algae and photosynthetic bacteria are unique among phototrophic organisms in their ability to fix nitrogen and yield biomass exceptionally high in protein content. Some strains of nitrogen fixing marine blue-green algae tested in our laboratory exhibited protein concentrations in excess of 60% of dry weight with significant quantities of essential amino acids. Nitrogen fixing blue-green algae were already successfully used as biofertilizer in other laboratories.[71,72,74,75]

## CONCLUSION-GENERAL TRENDS

The research summarized above includes concepts which could be applied in the near future, like blue-green algal fish feed and photosynthetic bacterial hydrogen production from waste. Others may not reach fulfilment for some years to come, like biological hydrogen production from water. Nevertheless, all of these proposed technologies are aimed at areas of eminent need. Future advances and ultimate success of these technologies, will depend on factors such as the research funding availability.

The question of how biological solar energy technologies will be successfully implemented is a complex interaction of successful design, demand, and economic issues. Over the past few years there has been a shift in interest away from the utilization of photosynthetic microorganisms for food production to chemical, medicine and fuel production. Production technologies for polysaccharides, lipids, glycerols, carotenoids, hydrocarbons, biologically active substances, enzymes, ethanol, methane and hydrogen by photosynthetic microorganisms, are being developed. Mass culture of these organisms in waste water from industry and food processing factories, is being developed. Certain new approaches and technologies, like genetic engineering, may hasten the development in future. The coupling of the production of the above substances, with the simultaneous environmental clean-up will have greater impact to society in the future. This can optimize the economics of these systems also. In the near-run "a multiple utilization" approach through the utilization of biological solar energy systems,[28,33] should be considered on an economic, ecological and energetic standpoint. As the price structure of fossile fuels changes in the future years, the picture may be altered, and wider range of application will become feasible.

Another important issue to keep in mind is the regional differences in the suitability of alternative technologies.[32] There is a tendency for researchers, in the highly industrial-

ized nations like the U.S.A. and Japan, to judge the merit of systems on a high intensity demand basis. There are, however, many biological solar energy technologies which make only a small contribution to the total energy budget of the world, but have a major impact on the quality of life for many people not only in the industrialized countries, but also in the developing countries.

The task of developing viable resource production systems based on algal and photosynthetic bacterial cultures must be looked at from a global perspective.

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